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EVIDENCE FOR THE EVOLUTION OF PHYTOPATHOGENIC VIRUSES FROM MITOCHONDRIA AND THEIR DERIVATIVES. I. CYTOLOGICAL AND GENETIC EVIDENCE[1]

M. W. Woods and H. G. DuBuy

(Accepted for publication January 11, 1943)

CYTOLOGICAL AND GENETIC EVIDENCE

INTRODUCTION

All plant viruses that have been chemically isolated contain d-ribose nucleoprotein (1). Some of these viruses at least are monomolecular in nature. At present, there is divided opinion as to the origin of viruses. Whereas most workers in the past have inclined to the view that viruses are derivatives of parasitic microorganisms, there has long been a school holding that these entities are autocatalytic proteins of ultimate host-cell origin, although the exact proteins have never been clearly defined. Thus, Woods (20) proposed the enzymic theory, which was further advanced with modifications by Freiberg (9), and Vinson (19). In 1933 Vinson demonstrated that the virus of tobacco mosaic is protein in nature. Stanley (18) later reported the isolation of this protein in "crystalline" form. Bawden *et al.* (2) were the first to demonstrate the nucleoprotein structure of viruses. In a previous paper (21) we demonstrated a relationship between the chromoprotein complex of the plastids of normal cells and tobacco-mosaic virus nucleoprotein. In the following two papers (*cf.* 7) we present proof that a ribonucleoprotein is a part of the normal chromoprotein complex. Furthermore, by presenting cytological and chemical evidence that characteristics of plastid-controlled variegations are intermediate between those of normal plants and virus-diseased plants, we connect virus proteins phylogenetically, over the variegation-inducing agents (abnormal plastids or chondriocontes), with proteins of the normal plastids or chondriocontes.

The similarities in symptoms of certain plastid-controlled variegations and certain infectious mosaic diseases are often striking. In both types of disease the symptoms are not limited to the chlorophyll alone, but as Woods (20) and Davis *et al.* (6) have shown, other physiological processes are involved. Woods believed both types of disease to be fundamentally alike; a contention borne out by the present work. Once plastids have mutated, such mutated plastids cause leaf variegation (5, 6, 12, 17). As Guilliermond (10) and others have pointed out, the plastids of higher plants can be considered as differentiations of the chondriome. The term chondriome includes all mitochondrial elements of the cell and their derivatives. Thus the

[1] Scientific Paper No. A42, Contribution No. 1869 of the Maryland Agricultural Experiment Station (Department of Botany).

TABLE 1.—*List of investigated variegated species and clones arranged in approximate order of their position in the spectrum (cf. Fig. 1)*

Variegated species and clone	Symptom Type[a]	Location in spectrum[b]	Homochondric variegated cells				Heterochondric cells		Graft transmission	Origin of clone
			V.-i. plastids			Approx. reduction in avg. cell size	Inhibiting action of v.-i. plastids or chondriocontes on normal plastids	Approximate reduction in cell size		
			Approx. size ratio normal/V.-i.	Color in immature cell	Color in mature cell					
Weigela sp.	P, S	I A	1.0/1.0	Lt. green	Lt. green	15% or less	Appar. absent	.....	Not tested	Hort. var.
Lonicera japonica Thunb. clone #2	P, S	I A	1.0/1.0	Lt. green	Lt. green to yellow	None	Do	.....	Do	Field
Ligustrum vulgare L.	P, S	I B	1.7/1.0	Lt. green	Lt. green to yellow	None or very slight	Do	.....	Do	Hort. var.
Anthirrhinum majus L. clone #1	P, S, trace MS	I B	1.7/1.0	Lt. green	Lt. green to yellow	None or very slight	None to very slight	Ap. none	Do	Spontaneous
Capsicum annuum L.	S	I C	2.0/1.0	Lt. green	Lt. green to yellow	None or very slight	Not determined	.....	Do	Spontaneous
Xanthium sp. (Tourn.) L. clone #1	S, MS	II A	1.3/1.0	Lt. green	Yellow	Do	None to slight	None or very slight	Do	Field
Lonicera japonica Thunb. clone #1	S, ext. MS	II B	1.5/1.0	Lt. green	Yellow	None	None to very slight	None	Negative	Field
Trifolium pratensis L.	S, MS	II C	2.0/1.0	Lt. green	Yellow to colorless	17% or less	Pronounced	15% or less	Not tested	Field
Ambrosia trifida L.	S, ext. MS	II C	1.5/1.0	Lt. green	Yellow	None or slight	Slight to pronounced	None or slight	Negative	Field

TABLE 1—(*Continued*)

Variegated species and clone	Symptom Type[a]	Location in spectrum[b]	Homochondric variegated cells				Heterochondric cells		Graft transmission	Origin of clone
			V.-i. plastids			Approx. reduction in avg. cell size	Inhibiting action of v.-i. plastids or chondriocontes on normal plastids	Approximate reduction in cell size		
			Approx. size ratio normal/V.-i.	Color in immature cell	Color in mature cell					
Antirrhinum majus L. clone #2	P, S, MS	III A	2.0/1.0	Pale yellow	Colorless	15% or less	Not determined	.....	Negative	Spontaneous
Rubus occidentalus L.	S, ext. MS	III A	3.0/1.0	Pale yellow	Colorless	None or very slight	Not determined	.....	Not tested	Spontaneous
Xanthium sp. (Tourn.) L. clone #2	S, ext. MS	III A	3.0/1.0	Pale yellow	Colorless	20% or less	Slight to pronounced	20% or less	Not tested	Field
Opuntia sp. Mille	P, S, MS	III A	1.2/1.0	Pale yellow	Colorless	None or very slight	Not determined	.....	Not tested	Horticulture
Hedera helix L.	Nearly P, S, MS	III A	1.7/1.0	Pale yellow to colorless	Colorless	10% or less	Pronounced	10% or less	Negative	Horticulture
Peperomia sp. Ruiz and Pav.	P, S, MS	III B	3.5/1.0	Pale yellow	Colorless	None or slight	Pronounced	None or slight	Not tested	Horticulture
Cucumis melo L.	S	IV A	Extremely small to chondriocontal	Colorless	Colorless	25% or less	Not determined	.....	Not tested	Spontaneous?
Euonymus radicans Sieb. clone #1	P, S, MS	IV B	Chondriocontal	Colorless	Colorless	30% or less	Very pronounced	30% or less	Positive	Horticulture
Lonicera japonica clone #3 (= *Lonicera brachypoda reticulata* Nichols)	Vein-yellow	V	Subchondriocontal units. Apparently homochondric variegated cells cannot exist.			.....	Very pronounced	None or very slight	Positive	Horticulture
Nicotiana tabacum L.	MS	VI	"	"	"	.....	Slight to very pronounced	Slight to very pronounced	Positive	Mechanically infected plants

[a] P = periclinal; S = sectorial; MS = Mosaic-sectorial.
[b] Capital letters indicate relative positions within the regions of the spectrum.

proplastids of the embryonic cell are considered as specialized mitochondria ("active chondriosomes" or "chondriocontes" *sensu* Guilliermond). Since chondriocontes have a definite heredity and are capable of mutation, they have fundamental properties in common with viruses. These properties of chondriocontes are probably under control of the ribose nucleoprotein component, which we have isolated from the normal plastid. The present paper furnishes cytological and other evidence for the origin of phytopathogenic viruses from the chondriome.

MATERIALS AND METHODS

Variegated clones of the various species investigated were obtained from greenhouse stocks or collected in the field[2] (Table 1). Whenever possible, clones were established from both green and variegated shoots of the same plant species, and, where possible, also from the same plant. In many instances it was possible to compare normal and variegated tissues in the same leaf. Plants were grown in the greenhouse in sand culture and supplied with appropriate nutrient solutions. Material for microscopic study was aspirated in freshly prepared 10 per cent neutral Formalin in distilled water, and sectioned with a razor. This fixative preserved the nuclei and cytoplasmic structures in a manner closely resembling their appearance in living cells. Living cells, prepared in dilute nutrient solution, were compared with fixed material. An aqueous solution of iodine in potassium iodide was the most satisfactory stain for mitochondria and plastids. In some instances Janus green B was used as a vital stain for mitochondria. Grafts and bud transfers were made with techniques appropriate to the plant material involved.

EXPERIMENTAL

Symptoms of Variegation

Symptom patterns in variegated leaves were either periclinal, sectorial, or mosaic sectorial, (Fig. 1, Fig. 8, A). In the sectorial types varigated areas alternate or form a mosaic with normal tissues, whereas in the periclinal types the normal tissues occur as a central core surrounded by variegated cells or *vice versa*. In many plants the onset of variegation is characterized by sectorial variegation, which changes to a stabilized periclinal form maintained by vegetative propagation (Fig. 8, B). Massey (17) has provided an explanation for this conversion to the periclinal form on ontogenetic grounds. Certain variegated clones, however, remain mosaic-sectorially variegated (*e.g.*, *Lonicera japonica* (Fig. 1, region II) and *Xanthium* clone 2) (Table 1). In these variegations the symptoms most closely resemble those occurring in such infectious diseases as *Abutilon* and tobacco mosaic. Some variegations such as those occurring in *Euonymus* may continue to develop all

[2] We acknowledge the aid rendered by Miss H. M. Christensen, Mr. C. E. Cox, Mr. R. Degen, Mr. J. Haney, Dr. J. B. S. Norton, Mr. W. L. Smith, Mr. R. Stewart, Mr. D. L. Stoddard, Dr. F. L. Wellman, and Mr. I. S. Zalph in collecting many of the clones investigated.

three major symptom types. As will be shown below the symptom pattern of variegation depends largely upon the behavior of the abnormal chondrioconte or plastid during leaf ontogeny.

HISTOLOGY AND CYTOLOGY OF VARIEGATED TISSUES

Histology

The symptom pattern of variegation can generally be explained on the basis of distribution of variegation-inducing chondriocontes[3] during division

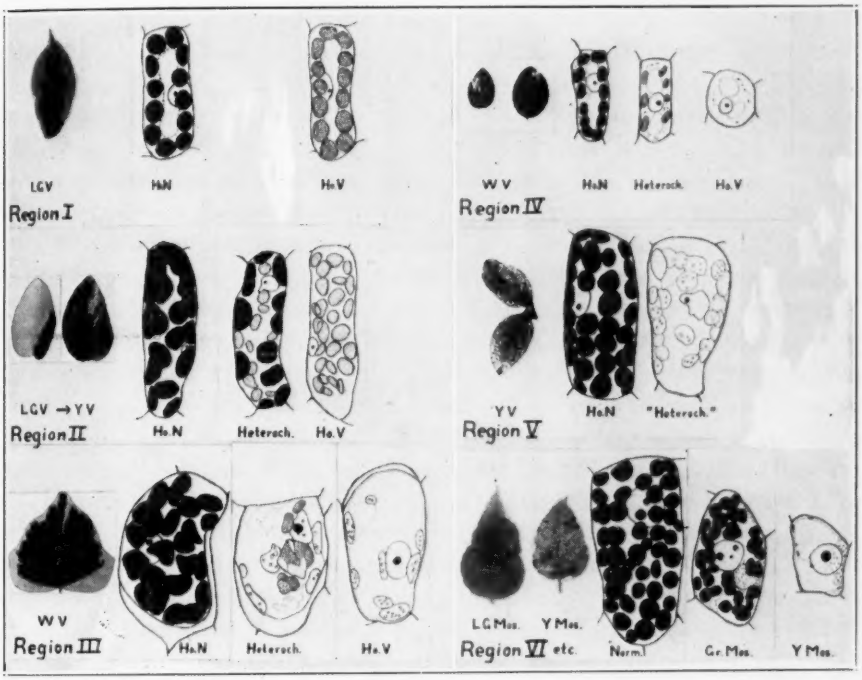

FIG. 1. Six types of variegations or viroses, representing 6 regions of a spectrum of variegation. Successive regions are based on progressively more drastic modifications in the variegation-inducing plastid (loss of pigments, loss of normal function, and reduction in size and chemical complexity). In each case photographs of leaf symptoms are accompanied by camera-lucida drawings (×2000) of cells with normal plastids only (homochondric-normal = Ho.N), cells with both normal and variegation-inducing plastids or chondriocontes (heterochondrie), and cells with variegation-inducing plastids or chondriocontes only (homochondric-variegated = Ho.V). In region VI a noninfected tobacco cell is shown together with a light green mosaic-infected and a yellow mosaic-infected cell. LGV = light green variegation; YV = yellow variegation; WV = white variegation. Illustrative species are as follows: region I, *Weigela* sp.; II, *Lonicera japonica* clone 1; III, *Hedera helix;* IV, *Euonymus radicans;* V, *Lonicera japonica* clone 3, and VI, *Nicotiana tabacum* var. Turkish, light-green and yellow tobacco mosaics (*Marmor tabaci* H.).

of pre-existing normal or variegated mother cells, and can be interpreted on a chimaeral basis. The composition of the mesophyll varied considerably in different parts of the same leaf or in different leaves of the same clone. Certain mosaic-sectorial patterns, however, could not be interpreted ade-

[3] Hereafter the term v.-i. will be substituted for the expression "variegation-inducing."

quately on a simple chimaeral basis. These were observed, for example, in *Lonicera japonica* clone 1 *Xanthium canadense* clone 2, and particularly in *Enonymus radicana* clone 1 (*cf.* Fig. 1).

Normal Cells (*Homochondric Normal Cells*). In normal cells (Fig. 1, Ho.N) the chloroplasts appeared as flattened spheres containing many minute pigment-bearing discs (grana) imbedded in a colorless stroma. The chloroplasts usually formed a single layer with their broad axes parallel to the cell wall, all available space being occupied. Starch grains could readily be delineated with IKI.

Completely Variegated Cells (*Homochondric Variegated Cells*). In completely variegated cells (Fig. 1, Ho.V) the variegation-inducing plastids were generally smaller than the chloroplasts of the normal cells, and often more numerous per cell and *light green in color*. The range of variation is summarized in table 1. In mild variegations (Fig. 1, region I of the "spectrum") the plastids of the mature cells remained green and were occasionally reduced in size. Distinct grana were sometime present, but starch grains were usually small or lacking. Cell size or shape were not markedly affected in most cases. In more extreme variegations (Fig. 1, regions II to III of the "spectrum") the pigment content of the v.-i. plastids was further reduced, as was their size. The cell size was often affected. The variegated cells contained more plastids than normal cells. For instance in *Lonicera japonica* clone 2, in which the cell size does not change the average number of normal plastids per unit cell area (32 cells measured) was $14.3 \pm .3$. In variegated cells (28 cells measured) the number of vi-i. plastids was $16.0 \pm .4$ per unit area. The fact that homochondric variegated cells contain more plastids than the corresponding normal cells indicates a higher division rate for the v.-i. plastids. The plastids of cells sometimes contained a small amount of chlorophyll (Fig. 1, region II), although in mature cells they were either *yellow or colorless*. In very old cells the v.-i. plastids were generally colorless and often highly vacuolate (Fig. 2). In the most extreme variegations the chondriocontes failed to develop beyond the mitochondrial stage and were always without pigment (Fig. 1, region IV). In these cases cell size and shape were generally markedly affected. In the types of variegation of regions II to IV of figure 1 changes in cell physiology were further evidenced by increased sensitivity of the tissues to adverse environmental conditions (mechanical and chemical injuries, desiccation, etc.), and development of vacuolar anthocyans. Abnormal vacuolation of the cytoplasm in old cells, and increased oxidase activity as indicated by browning of the cells of young leaves were observed. *In general the picture of cell pathology became more virose-like in proportion to the increasing abnormality of the v.-i.-inducing plastids or chondriocontes* (*cf.* 8). This was particularly marked in cells containing *both* variegation-inducing and normal plastids. These will be referred to as heterochondric cells.

Partly Variegated or Heterochondric Cells. Heterochondric cells have not been observed in some of the mildest variegations (Table 1, region I). In

some of the variegations of region I and in all of region II the v.-i. plastids did not cause marked pathological changes in the normal chloroplasts of the heterochondric cells. However, as the cell aged the normal plastids occasionally became lighter green. As in completely variegated cells, heterochondric cells with a high proportion of v.-i. plastids often showed evidence of physiological abnormality.

Heterochondric cells in more extreme variegations (Fig. 1, regions III to IV) are interesting because they most closely resembled cells infected with

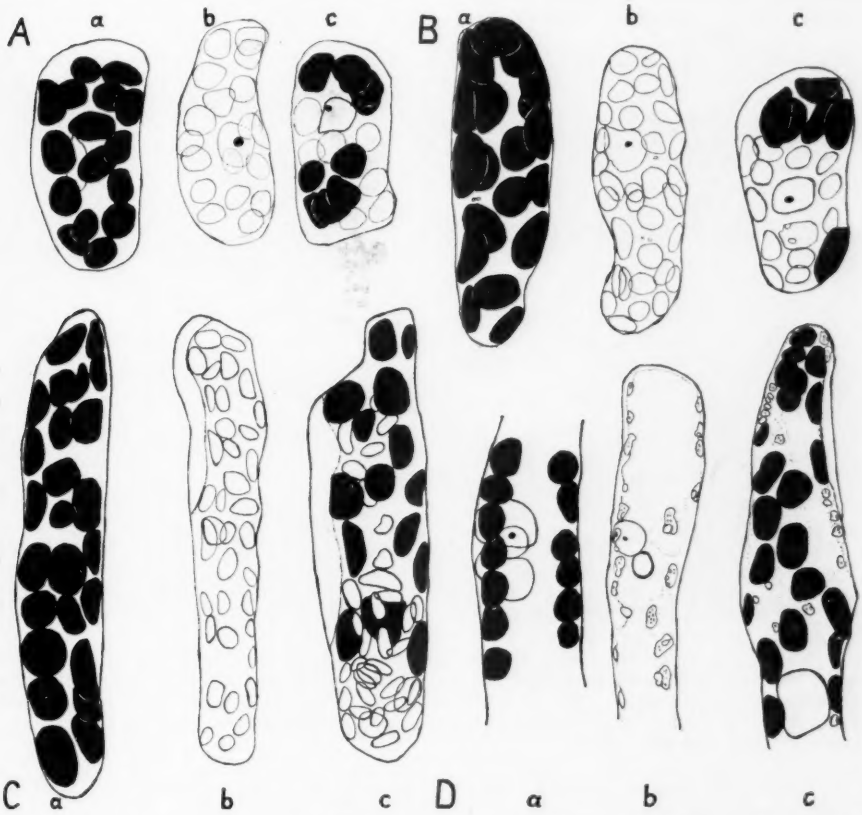

FIG. 2. A to D. Successive developmental stages of homochondric normal cells (Aa, Ba, Ca, Da), homochondric variegated cells (Ab, Bb, Cb, Db), and heterochondric cells (Ae, Be, Ce, De) of *Lonicera japonica* clone 1. In this variegation the v.-i. plastids do not cause marked changes in the normal type plastids of heterochondric cells. Note progressive reduction in size of the v.-i. plastids with age of cell. Differences in cell size in this case are not significant.

"typical viruses." In *Xanthium* sp. clone 2, for example, the v.-i. plastids in the mature cell were colorless and rarely contained starch (Fig. 3, C). In heterochondric cells from mosaic-sectorial areas marked reduction in the size, the chlorophyll content, and the starch content of the normal chloroplasts was sometimes noted. Cell size also was often reduced. Adjacent cells, which contained only normal plastids, always appeared entirely

normal. The extent of derangement in the normal plastids of heterochondric cells was in proportion to the relative number of v.-i. plastids present in the same cells. It is significant, however, that in *Xanthium* sp. clone 2, and in certain other species, the occurrence of even a large proportion of v.-i. plastids in heterochondric cells did not always result in visible derangement of the normal chloroplasts. In this respect the behavior of the v.-i. plastids parallels the action of certain viruses in that the presence of the virus may or may not, depending on conditions of cell metabolism, cause visible changes in the chloroplasts of infected cells.

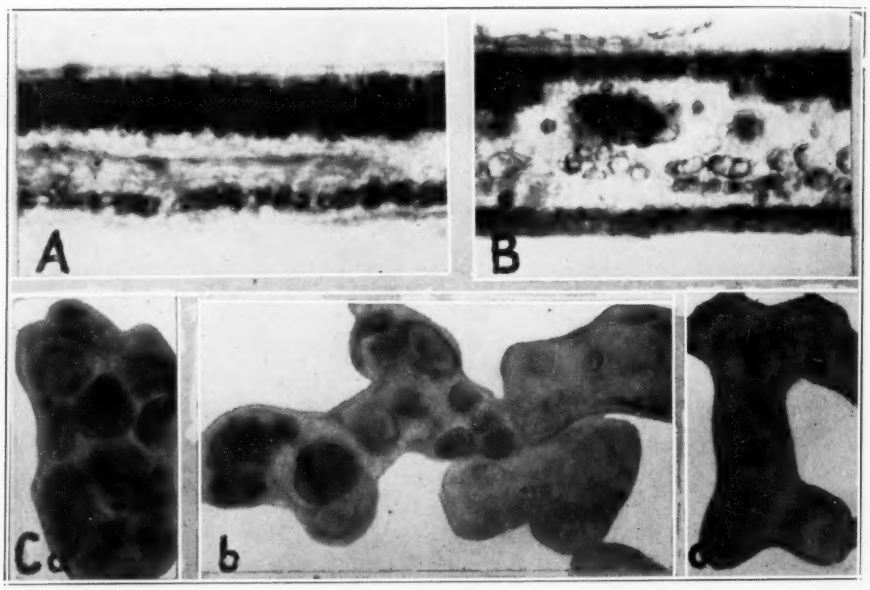

FIG. 3. A. *Lonicera japonica* clone 1. Sector of leaf with central mesophyll made up of homochondric-variegated cells. B. *Euonymus radicans*. Central mesophyll of homochondric-variegated cells. (Note similarity to A.) C. *Xanthium* sp. clone 2. a, homochondric normal cell; b, heterochondric and adjacent homochondric-variegated cells; c, homochondric variegated cell.

The variegation-inducing chondriocontes of the most extreme variegations (table 1, region IV), exert, like certain viruses (4, 8), a very strong inhibitory action on the normal chloroplasts occurring in the same cell. Thus, in white variegated clones of *Euonymus* (Figs. 4 and 5), the v.-i. chondriocontes, which never developed beyond the mitochondrial stage, completely inhibited the development of the normal plastids in the embryo cell. In such cases the maturation of the whole cell was markedly affected.

EVIDENCE FOR INTERCELLULAR MIGRATION OF V.-I. CHONDRIOCONTES

Transition Areas

In some of the rapidly growing immature leaves of *Euonymus radicans* clone 1, certain mosaic-sectorially variegated areas contain light-green cells

that form a transition zone between the normal-green and completely variegated tissues. These light-green transition zones contain cells with varying degrees of chloroplast inhibition, degeneration, or both (Fig. 1, Region IV, Heteroch.). There is likewise a gradation in degree of inhibition in cell growth (Table 2). The most severely affected cells occur nearest the com-

TABLE 2.—*Shape of palisade cells in transition zone between normal green and completely variegated (white) areas in a young leaf of Euonymus radicans. Note relationship between condition of the chloroplasts and shape of cell*

Cell type	Dimensions of cells			
	Length	Width	Length/width	No. measured
Normal cells: normal chloroplasts	4.66	1.52	3.06	22
Cells with degenerating chloroplasts	4.49	1.68	2.67	16
Cells without chloroplasts	3.24	2.32	1.39	18

pletely variegated tissues. Presumably, similar transition zones have been described by Hein (12) for *Scirpus, Diffenbachia, Dracaena, Croton, Abutilon* and *Plantago* sp. as gradual degeneration of the plastids on the margins of the discolored areas. These transition zones with heterochondric cells in *intermediate* stages of chloroplast breakdown and growth inhibition (Fig. 4) can best be interpreted by assuming that they were *invaded by v.-i. chondriocontes at some post-embryonic stage of ontogeny.* If these cells had been heterochondric from the beginning, cell size and shape would have been different from normal cells. No transition zone could be found in these cases (Fig. 5). The fact that this variegation, which shows transition zones,

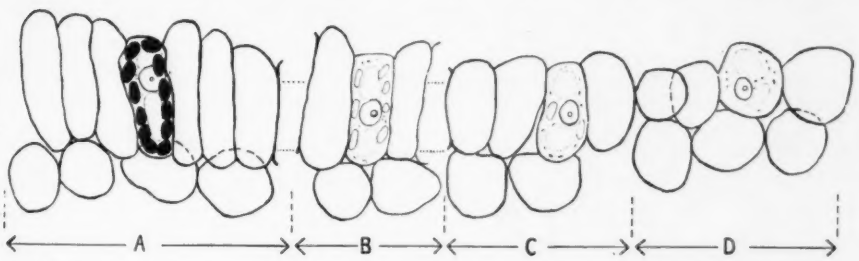

FIG. 4. Various cell stages of the transition zone of a mosaic-sectorial variegation of *Euonymus radicans:* A, normal cells; B, heterochondric cells; C, heterochondric cells with strongly degenerated "normal" plastids; D, totally variegated cells. (Semidiagrammatic camera-lucida drawing.)

has been graft-transmitted supports this view (Figs. 4 and 5). It should be emphasized, however, that intercellular migration of v.-i. chondriocontes in this plant seems to be very limited as most of the symptom pattern is clearly of the chimaeral type encountered in the other "non-transmissible" variegations (Fig. 3B and 5). Indications of intercellular migration of v.-i. chondriocontes in certain mosaic-sectorial variegations of the less severe type (Regions II and III, Fig. 1) were found, but the cytological evidence could be interpreted with less certainty. *Lonicera japonica* clone 1 (Figs. 3, A,

and 6) was the most favorable object for these studies, since both normal and v.-i. plastids could be distinguished readily in the heterochondric cells. In this case they exerted little or no degenerative action on the normal chloroplasts of mature cells. However, the inability to use changes in cell size as a "marker" for determination of the time of invasion was a disadvantage. In this clone the chief indications of limited intercellular migration were the occurrence of small isolated irregular patches or islands of normal cells surrounded by a diffuse transition zone of heterochondric cells in otherwise completely variegated areas. Furthermore, there existed a pronounced tendency for masses of both heterochondric and homochondric variegated cells to be delimited by the leaf veins. In the first case the highest frequency of v.-i. plastids occurred in the transition zones nearest the surrounding com-

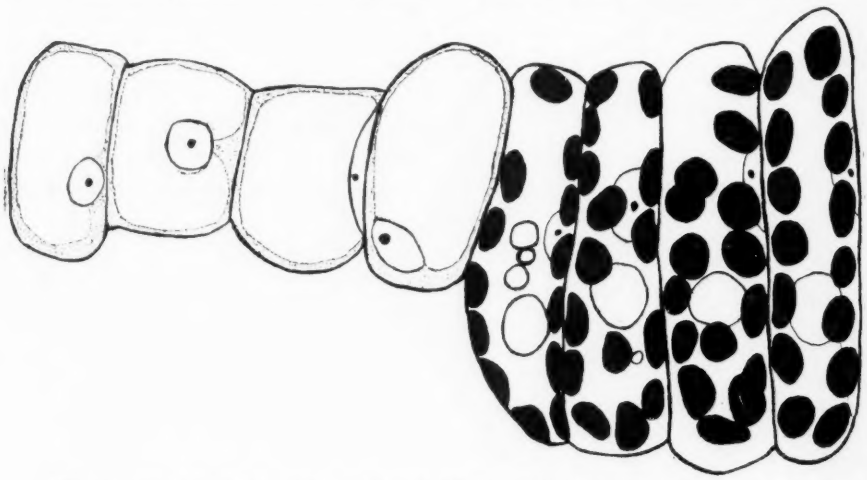

FIG. 5. Sharp separation of normal and totally variegated cells of a leaf of *Euonymus radicans*. Notice difference in cell size. (Semidiagrammatic camera-lucida drawing.)

pletely variegated tissues, indicating that progressive invasion into the normal cells during early ontogenetic stages had occurred. Since vascular tissues are differentiated very early it seems likely that migration of v.-i. chondriocontes may occur after the formation of the vascular strands, *but before maturation of the leaf,* since there is no extension of the variegated area in the mature leaf.

The establishment of v.-i. chondriocontes in normal cells seems to depend on the ability of the invading chondriocontes either to inhibit normal plastid development (as occurs in *Euonymus*) or on their ability to prevent further division of the normal chondriocontes or plastids, which are already present at the time of invasion. Whether daughter cells will be completely variegated may, then, depend on either segregation of normal and v.-i. chondriocontes or on early invasion followed by inhibition of the development of the normal plastids. The simplest explanation for those cases where new growth of certain variegated shoots becomes *progressively* more variegated and more mosaic-like is the occurrence of some invasion. These signs of invasion were

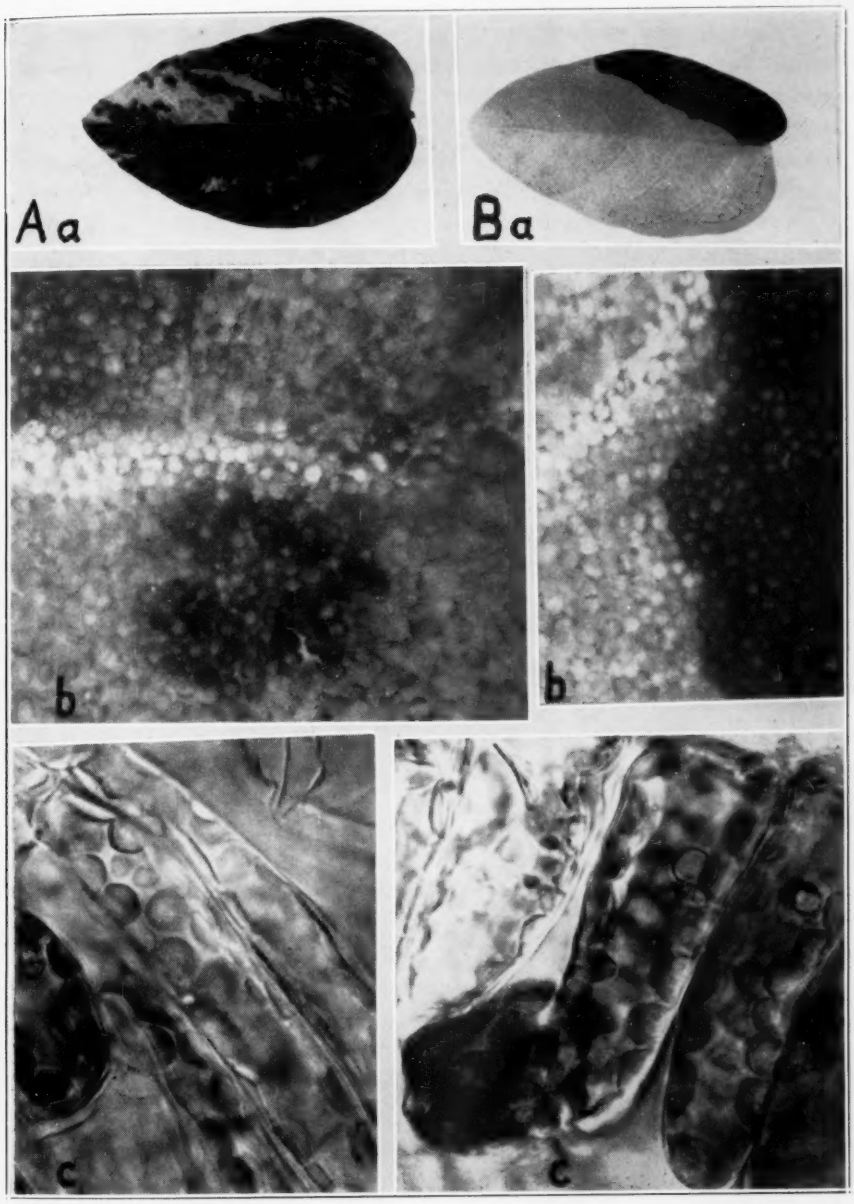

FIG. 6. *Lonicera japonica* clone 1: Aa, mosaic-sectorially variegated leaf; Ab, a mosaic sectorial area, × 400; Ac, section showing homochondric normal, heterochondric and homochondric variegated cells, × 2000. Note difference in size of normal and v.-i. plastids. Ba, sectorially variegated leaf; Bb, a sectorial area; Bc, adjacent homochondric normal and homochondric variegated cells. (Magnifications as under A.)

never observed in variegations of region I: these always showed relatively regular patterns of variegation. The signs of invasion become more pronounced the farther one proceeds in the spectrum. Thus in *Lonicera japonica* clone 2 (Region IA) the v.-i. plastids are slightly abnormal and the symptom pattern is still mainly regular. In *Lonicera japonica* clone 1 (Region IIB) as we have seen, the differences between the two type plastids are already more pronounced and mosaic patterns of variegation occur. The same is true for *Xanthium*. The most extreme case is that of *Lonicera japonica* in which the v.-i. agent is apparently invisible and transmission general.

In general, there seems to be a correlation between the degree of abnormality of the v.-i. chondriocontes or plastids (as indicated by size, color, or both) and their invasiveness as indicated by a mosaic pattern. *However, a small chondrioconte is not necessarily invasive* (see p. 645). Furthermore, the smaller the v.-i. chondrioconte, the stronger its effect upon the normal plastid. In other words, in order to invade, the v.-i. chondrioconte has to be small. Since small v.-i. chondriocontes have a strong effect upon the normal plastids, invasion, when it occurs, is always linked with the more pronounced changes of the normal plastids.

GRAFT TRANSMISSION OF VARIEGATION

Graft transmission of chondrioconte-controlled variegation would afford direct proof of the ability of v.-i. chondriocontes to migrate intercellularly. So far in the present investigation graft-transmission studies have been limited to 5 species. The results indicate that, while graft-transmission of typical chondrioconte-controlled variegation can occur, such transmission is extremely difficult to accomplish. Thus in 10 attempts to transmit mosaic-sectorial variegation of *Lonicera japonica* clone 1, there was no evidence of transmission over 10 months after grafting. Three attempts to transmit the variegation of *Hedera helix* (Fig. 1), and 5 attempts to transmit a mosaic-sectorial variegation of *Ambrosia trifida* (Table 1) also failed although the variegated coins grew well for several months after grafting. The vein-yellowing variegation of *Lonicera japonica*, *L. brachypoda reticulata* Nichols of horticulture (14, 16) (Fig. 1), however, was graft-transmitted in more than 10 cases to several green varieties of *L. japonica*. The first symptoms usually developed on the stock plants 30 to 60 days after grafting. Movement of the variegation-inducing agent (as judged by symptom response) was through the vascular system. Secondary graft-transmission from graft-infected plants was readily accomplished. Plants infected 10 months earlier grew vigorously and are continuing to show symptoms on new growth.

Eight attempts to transmit the white mosaic-sectorial variegation of *Euonymus radicans* to *E. japonica* were made. Observations have been continued for over 200 days, but no evidence of successful transmission has occurred. However, of 6 attempts to transmit this variegation to green

clones of *E. radicans* 1 was positive. While the successful transmission involved only 1 plant, the evidence is entirely conclusive, as detailed records were made of the plant several months before and after grafting. The

TABLE 3.—*Graft-transmission of type IV variegation in Euonymus radicans. A single variegated shoot was grafted into the healthy stock plant*

Days after grafting	Condition of graft-inoculated plant			
	Number branches on whole plant	Variegated shoots	Per cent of branches variegated	Severity of symptoms
0	ap. 40	0	0	All healthy
77	56	8	14	Very slight
137	91	12	13	Slight to pronounced
274	121	21	17	Slight to pronounced

results are summarized in table 3. The ingrafted diseased cion was about 3 cm. long and bore several heavily variegated leaves. Good union was secured between a nodal region of the cion and a sub-terminal node of a rapidly growing shoot on the healthy stock plant. The first symptoms were observed on the latter 77 days later in 8 separate branches of about the same age. Seven of the infected shoots bore one or two leaves each with small flecks of variegated tissue (Fig. 7, A, B). The apical meristems were

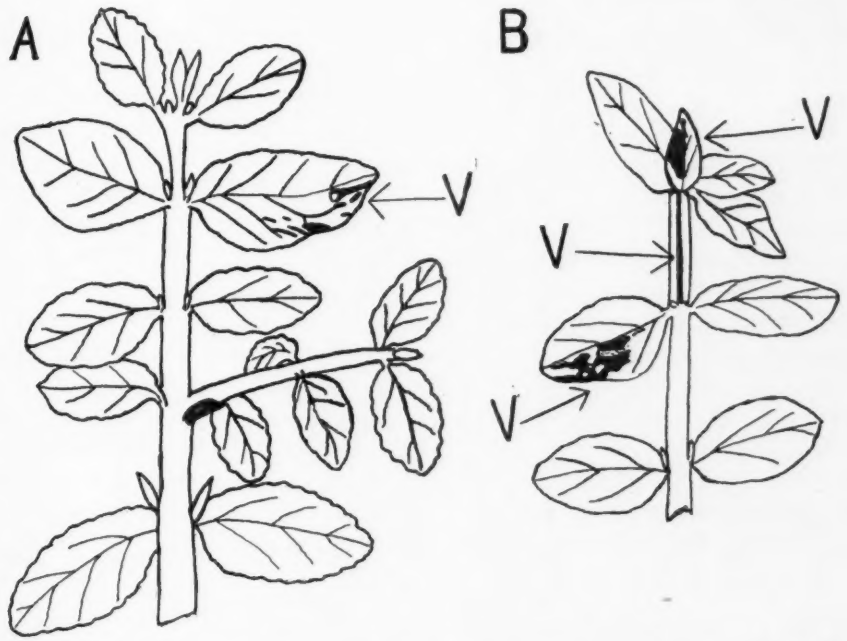

FIG. 7. Two shoots of *Euonymus radicans*, showing veriegated areas 77 days after transplanting a white mosaic-sectorially variegated shoot of the same species: A, leaf variegation only; B, leaf and apical meristem variegation.

not involved, except in the eighth shoot (Fig. 7, B). That the symptoms in each of these 8 branches were primary was evidenced by the complete absence of symptoms anywhere else on the plant at this time. The infected stem tip on the eighth branch was separated from the original ingrafted variegated cion by 35 nodes, or a distance of approximately 69 centimeters, The v.-i. agent presumably moved this distance through the vascular channels and then entered the parenchymatous cells of the leaf primordia or stem tip. It gradually continued to spread to other parts of the plant entering the apical meristems of several branches. The tendency for the v.-i. chondriocontes to remain localized is demonstrated by the subsequent behavior of apparently symptom-free cuttings, which were removed from the infected plant. Thus, 4 variegated cuttings removed 95 to 170 days after grafting produced only variegated plants in which 17 of a total of 29 branches were variegated, some heavily. Five green cuttings removed 92 to 152 days after grafting produced 3 variegated plants with 7 out of a total of 17 branches variegated (only traces of variegation in some), and 2 non-variegated plants with a total of 17 branches. On the original infected plant some very rapidly growing shoots have apparently grown away from the variegation. Similar localization of the virus of Abutilon mosaic and slow-moving strains of tobacco mosaic have been reported (15). In all of the variegated branches of the infected plants the symptoms are mosaic-sectorial or sectorial. Thus distribution of v.-i. chondriocontes in leaf parenchyma is chiefly by cell division, even though they may move long distances through the vascular tissues.

SEXUAL INHERITANCES OF VARIEGATION

Studies of sexual inheritance of v.-i. chondriocontes were limited chiefly to *Antirrhinum majus*. While the detailed results will be published later by Miss J. L. Showacre, certain data are presented here. The light-green variegated clone, and the white clone 2 (Table 1) were chiefly investigated. In both types the inheritance of the v.-i. chondriocontes proved to be matroclinous. Flowers produced on periclinally variegated shoots (Fig. 8) gave completely variegated progeny, which soon died. Flowers of sectorially-variegated shoots (Fig. 8, A), however, produced normal green, completely variegated, and sectorially variegated seedlings (Fig. 8, C). Many of the sectorially variegated seedlings possessed enough green tissue to complete their development. In other tests 22 seedlings obtained from a periclinally variegated *Lycopersicum esculentum* were completely variegated and died. In other words, it seems that continued sexual propagation of variegation depends upon maintenance of the mosaic sectorial pattern.

ORIGIN AND FREQUENCY OF VARIEGATION

Spontaneous origin of a recognized virus in a previously virus-free plant has never been proved. This is in harmony with a theory of their gradual evolutionary differentiation from chondrioconte nucleoproteins. The spon-

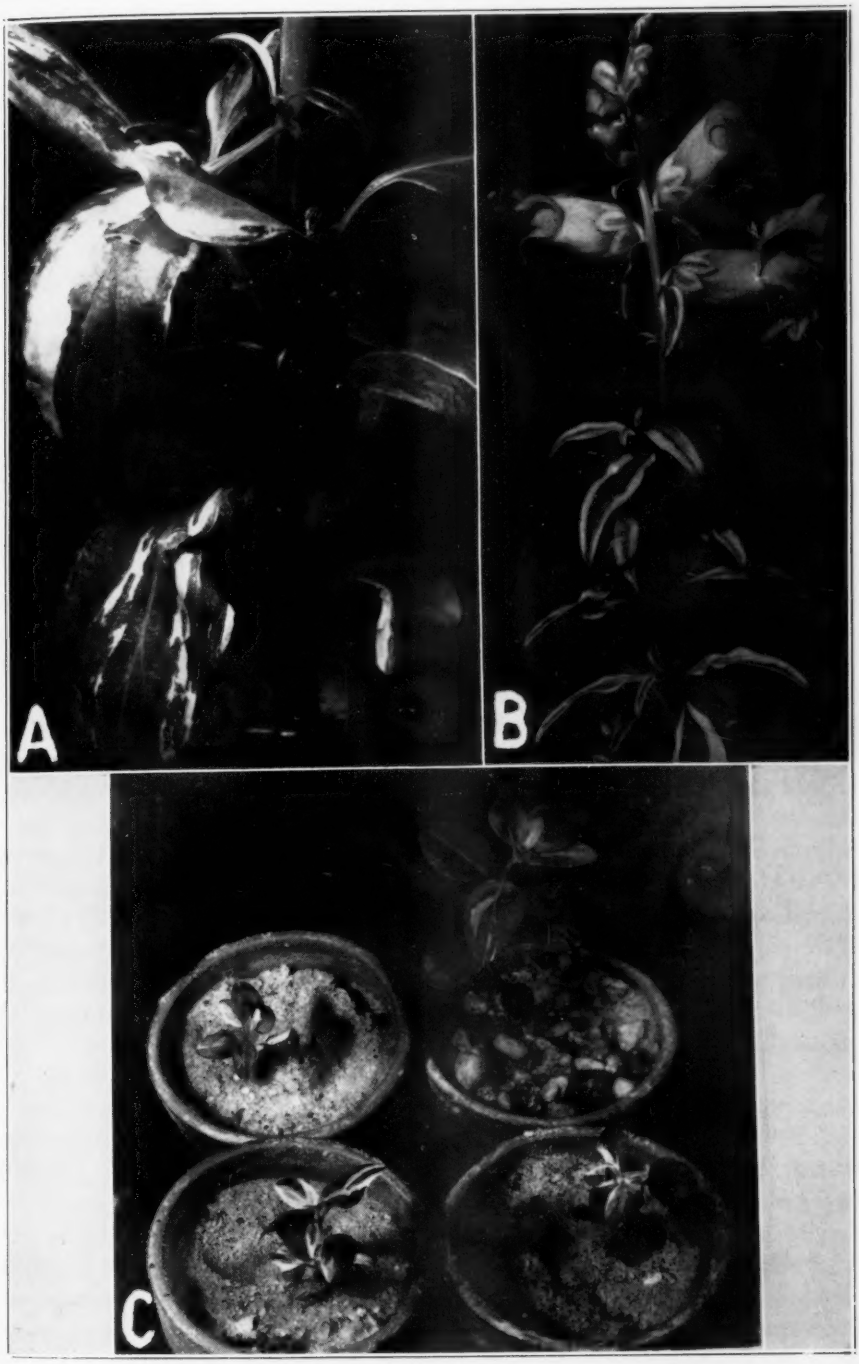

FIG. 8. A. Sectorially-variegated shoot, which produced sectorially-variegated seedlings (C). B. Pericinally variegated shoot which produced nonviable completely variegated progeny. (Courtesy of Miss J. Showacre.)

taneous development of chondrioconte-controlled variegations, on the other hand, has been observed in the present investigation. Thus the two variegated clones of Antirrhinum (Table 1) appeared in a progeny of approximately 1,000 seedlings from nonvariegated parents (we are indebted to Mr. F. B. Winkler for these clones). The variegated haploid clone of *Capsicum* (Table 1) arose as a single branch on the mother plant, which was over 6 months old. This haploid has been described in detail by Christensen and Bamford (3) from whom the variegated clone was obtained.

Field surveys of large populations of several species indicate that the frequency of variegation in nature is low (one in several thousand or million individuals), but more than enough to support the present theory. Approximate counts in selected regions of Maryland, for example, have shown that in *Ambrosia trifida* plastid mutation occurs relatively often (one in several thousand), whereas in *Xanthium* the frequency in most locations is much lower. One exceptional colony of *Xanthium* however, was found in which approximately 100 variegated individuals were observed in an area covering about one-half acre of ground. All of the variegated individuals but one were of the same white form. The one exceptional plant possessed light-green variegated plastids. Circumstantial evidence indicates that the variegated colony was produced by sexual reproduction. This is borne out by the occurrence of sectorially variegated seedlings in progeny obtained from 2 of these plants. So far only 2 mosaic-sectorial variegations have been found in *Lonicera japonica* (Table 1), although many thousands of plants have been examined. The vein-yellowing variegation in this species (Fig. 1) has been collected from 3 widely separated localities in Maryland, but each seems to represent an escape from an original clone that presumably traces back to a form long circulated in commerce (16). Also, over 20,000 black raspberry plants were examined in detail and only 2 cases of plastid-controlled variegation were found. In one locality over 3,000 plants of *Plantago lanceolata* were examined without finding a trace of variegation, although 2 sectorially variegated clones were found elsewhere. Information is rapidly accumulating indicating that almost any higher plant species may develop plastid-controlled variegation.

DISCUSSION

That certain variegations involving the chloroplast are to a certain extent under plastid control has often been indicated (5, 6, 12, 17). So far as the writers are aware, however, previous evidence for intercellular migration of plastids or proplastids is considered inadequate. Our study indicates that when such migration does occur it is while the plastids are in a chondriosomal condition. Intercellular passage of these chondriosomes or their essential constituents could probably take place through plasmodesmata. The concurrence of all conditions requisite for intercellular passage of chondriosomes or their essential constituents would be realized only at a critical stage in the ontogeny of the cell. This stage probably occurs before

maturity of the tissues. That the chief means of distribution of chondriosomes in the leaf tissues, however, is through cell division is obvious from the chimaeral structure of variegated leaves. The actual demonstration of intercellular passage of chondriocontes or their essential constituents is probably not possible with existing techniques, but strong evidence that such intercellular movement of chondriocontes really does occur has been obtained in the present study. The facts constituting this evidence cannot be explained by existing theories of chondrioconte segregation. They consist of the 3 groups of observations in favor of intercellular migration, which are presented here: (1) the manner in which heterochondric cells are distributed in the symptom pattern of certain types of variegations, (2) the varying degrees of inhibition of cell development of heterochondric cells in the transition zones, which sometimes occur between normal and variegated areas (Fig. 4), (3) the transmissibility by grafting. This evidence for limited invasion allows a further comparison of chondrioconte-controlled variegations and viroses.

The most important obstacle to the establishment of a connection between variegations and viroses was the difference in migration of the agent, which causes the abnormalities. A detailed survey of the field of plant viroses, however, reveals that they can be arranged in a series in respect to a progressively more pronounced invasiveness (13). Those that are the least invasive (*e.g.*, Euonymus- and Abutilon-mosaic viruses) are also those that produce symptoms more closely resembling certain chondrioconte-controlled variegations. Thus, again, this comparison constitutes further evidence for the evolution of viruses from variegation-inducing chondriocontes.

Since chondriocontes have definite powers of mutation and heredity, they are subject to natural selection. Furthermore, since the v.-i. chondriocontes can multiply in previously normal cells, and may retard or prevent the development of normal chloroplasts in those cells, it is obvious that the abnormal chondriosomal types are able to compete with the normal types for substrate. A similar competition between tobacco mosaic virus and normal plastids has already been reported (21). This constitutes a further parallelism of variegations and viroses.

A review of the data already published and of new data obtained in the present study indicates strongly that mutations within the chondrioconte, coupled with natural selection of more stable, actively multiplying, and invasive types may have resulted in the occurrence of such slowly invasive and probably unstable viruses as those causing Euonymus mosaic, Abutilon mosaic, etc., and through more extreme modification in viruses like the tobacco-mosaic virus proteins, which are very stable and highly invasive. These former viruses may still possess the complex coacervate lipoid-protein structure of chondriosomes. *Thus certain viruses may be considered as derivatives of the chondriome, which have become modified through evolution, rather than as derivatives of parasitic micro-organisms.* This agrees with Dufrenoy's observations that a virus that causes localized necrotic

spots but fails to become systemic is a virus that fails to induce systemic liberation of the protein fraction from the lipoprotein stroma of the plastids (Phytopath. Zeitsch. **5**: 290–301, Fig. 7), whereas a virus that becomes systemic causes the protein in the plastids to become remobilized into amino acids, such as tyrosin and leucine (*l.c.*, Fig. 8; also P. Z. **2**, p. 384, Fig. II). Chondrioconte-controlled variegations, therefore, are not necessarily virus diseases, although they may be potential starting points for the evolution of viruses, particularly in perennial plants. For instance, graft-transmissible variegations, such as that present in *Euonymus*, might be considered as proto-viroses.

Further evidence of evolution would consist in the demonstration that the chondriosomal or plastid protein complex of higher plants contains a ribose nucleoprotein of the same character as the known virus-ribo-nucleoprotein. This proof will be presented in another paper (7).

While the possible occurrence of drastic mutations in the chondrioconte nucleoprotein, leading directly to the formation of a "monomolecular" virus cannot be excluded, the failure to demonstrate the spontaneous origin of any such viruses indicates that a more gradual type of evolution has occurred.

SUMMARY

Certain plastid-controlled (chondriosome-controlled) variegations can be arranged into a series or spectrum, depending on the extent to which the plastid structure and function is modified. Consecutive regions of this spectrum show an increasing similarity to certain virus diseases.

In general, plastid-controlled variegations are characterized by typical sectorial or periclinal chimaeral structure in the variegated leaves. Many mosaic sectorial variegations occur, however, that suggest that variegation-inducing chondriosomes may actually invade normal cells by intercellular passage at some critical stage of ontogeny.

The variegation-inducing chondriosomes often behave like viruses with respect to their physiological action on the cell.

A variegation of *Euonymus*, that appears to be of the chondriosomally controlled type, was transmitted by grafting to a previously nonvariegated stock plant.

Variegations usually are inherited matroclinously, as are viruses.

It is suggested that viruses have been derived through mutation and natural selection from constituents of the chondriosomes rather than from preexisting parasitic microorganisms.

The spontaneous origin of variegation has been observed in several cases. Furthermore, the frequency of such plastid mutations in nature is sufficient to furnish a basis for the present theory.

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ROOT ROTS IN STORAGE OF DECIDUOUS NURSERY STOCK AND THEIR CONTROL[1]

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In Federal and State nurseries in the United States large quantities of numerous species of trees, shrubs, and vines have been grown annually for use in reforestation and in soil conservation. For the most part these were transplanted to their permanent locations in the early spring as 1-year seedlings. To make such seedlings available for spring planting, usually they are dug in the preceding fall and stored over winter in "heel-in" trenches outdoors or indoors in packing sheds, root cellars, or caves.

In outdoor heel-in beds, as well as in indoor storage in nurseries in the Upper Mississippi Valley, large quantities of nursery stock have deteriorated every year by molding and rotting of the roots. This is distinct from the stem rot reported by Gilman and Sproat[3] and was a more common cause of losses. In the winter of 1937–38 at an Iowa nursery the entire stock of black locust (*Robinia pseudoacacia* L.) in overwinter storage was lost from such injuries. At a Minnesota nursery the annual losses of black locust seedlings have equaled 20 to 50 per cent or more. Other species grown in nurseries, suffering from root rot in storage, were especially Osage orange (*Maclura pomifera* (Raf.) Schneid.), Russian mulberry (*Morus alba* f. *tatarica* (L.) Ser.), black walnut (*Juglans nigra* L.), wild plum (*Prunus americana* Marsh.), bur oak (*Quercus macrocarpa* Michx.), and tulip tree (*Liriodendron tulipifera* L.).

In the fall of 1938 a study was undertaken in an Iowa nursery to determine the cause of these losses. Plants of the species mentioned above and, in addition, green ash (*Fraxinus pennsylvanica* var. *lanceolata* (Berkh.) Sarg.), Siberian pea tree (*Caragana arborescens* Lam.), gray dogwood (*Cornus racemosa* Lam.), climbing bittersweet (*Celastrus scandens* L.), Siberian elm (*Ulmus pumila* L.), Tatarian honeysuckle (*Lonicera tatarica* L.), riverbank grape (*Vitis riparia* Michx.), and prairie rose (*Rosa setigera* Michx.), were subjected to a wide variety of storage conditions outdoors in heel-in beds of soil, sand, shingletow, and sawdust, mulched and unmulched, and indoors in packing sheds, root cellars, caves, and refrigerators. Indoors the plants were ricked or heeled-in in shingletow, sphagnum, or sand. In ricking (shelving) the plants were stored by the root-to-root method and

[1] This work was a cooperative undertaking between the Division of Forest Pathology, Bureau of Plant Industry; the Department of Botany and Plant Pathology, Iowa State College; and the Nursery Division of the Soil Conservation Service. This paper is based on a more detailed report by George Y. Young and Albert F. Dodge, "Storage Losses of Black Locust and other Deciduous Nursery Stock." Mimeograph, 43 pp. 1941.

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[3] Gilman, J. C., and B. B. Sproat. A *Fusarium* following frost injury of *Robinia*. Iowa Academy of Science Proceedings **43**: 101–106. 1936.

some by the root-out method, and the roots of some were protected and those of others were left bare. Where a protective medium for the roots was used, different levels of moisture content were used. Temperature and relative humidity of storage also were recorded. Similar series were replicated in a Minnesota nursery where losses usually have been severe and a Missouri nursery where losses seldom were serious.

DESCRIPTION OF THE ROOT DECAY IN STORAGE

One of the first symptoms of the root deterioration referred to above is a certain stickiness, noted on handling the affected roots. Later, they appear to be wet; water-soaked areas of a darker hue than the healthy areas then become manifest. The latter usually appear first on or near the root extremities. The water-soaked areas may appear, however, on any part of the root from the soil collar down. These lesions enlarge and may coalesce to form extensive affected areas. Later, desiccation and shriveling occur, and almost invariably a gray-white to pink mycelium covers the affected areas. When a partly shriveled and molded root is cut longitudinally, the area of decay is evident by the discoloration of the cortex. At a later stage of decay, when most of the root is molded, the cortical tissues become flaccid, and the cortex may be easily opened with the fingers without cutting. At or just prior to this stage of decay, the cortex tissues of the root become "thready" and may be separated into strands. Still later the entire root becomes soft and mushy. Only in very few cases has this condition of the root extended beyond the collar into the stem.

STORAGE STUDIES WITH BLACK LOCUST

One phase of the work undertaken at Iowa was a study of the relation of the time of lifting of stock to the incidence of decay in storage. Plants of black locust were lifted at intervals in late fall and stored, both indoors and outdoors, under various methods of storage. The results of this work are summarized in table 1.

Examination of the table will show that in the packing shed nearly all of the root rot occurred in plants lifted on Nov. 7 and Dec. 14 when tempera-

TABLE 1.—*Percentage*[a] *of root rot in black locust as related to date of digging, air temperature, and storage treatment. Iowa, 1939*

Date stock lifted	Temperature (°F.) on day of digging			Ricked in packing shed	Heeled-in		
					In soil		In shingle-tow outdoors
	Min.	Max.	7 p.m.		In root cellar	Out-doors	
Oct. 28	36	77	57	0.3	3.3	0.1	88.0
Nov. 4	40	44	42	.....	9.0	0.0	57.0
Nov. 7	26	30	28	13.3	.....	.....	.....
Nov. 12	42	64	44	0.0	2.9	0.0	73.0
Dec. 6	25	45	39	0.8	20.0	72.0	79.0
Dec. 14	20	33	20	27.3	100.0	100.0	100.0

[a] Based on examination of 200 plants in each case.

tures below freezing prevailed at the time the plants were being lifted, since the maxima for these two dates are 30° and 33° F., respectively. Also, all plants stored in shingletow heel-in beds, outdoors, regardless of the date of lifting, suffered heavily from root rot. In the soil heel-in beds outdoors in the case where 72 per cent of the plants rotted, large air pockets were found in the heel-in trench due to faulty heeling-in, which resulted in the roots of these plants being inadequately protected from mass movements of cold air within the trench. For plants lifted on Dec. 6 it is possible that at least part of the stock was lifted in temperatures below freezing.

For the greater part of the work undertaken in Iowa the plants were dug on Nov. 12, with the temperature considerably above freezing (Table 1). Results of examination of some 10,000 plants are given in table 2.

TABLE 2.—*Percentage of root rot in black locust as related to method of storage. Iowa, 1939*

Indoor storage, all methods of storage	less than 0.5
Outdoor storage:	
Heeled-in in soil	less than 0.1
Heeled-in in coarse sand, mulched	8.3
Heeled-in in coarse sand, nonmulched	27.0
Heeled-in in shingletow, mulched and nonmulched	100.0
Plants left in nursery rows for spring lifting	0.0

The data in tables 1 and 2 show: 1. All plants lifted in temperatures above freezing and stored in adequate storage suffered little or not at all from root rot; 2. All plants lifted in temperatures below freezing, that is, plants whose roots were exposed to temperatures below freezing, all suffered heavily from root rot regardless of the method of storage; and 3. All plants stored outdoors in porous media, such as shingletow or coarse sand, that is, media that permit mass movements of cold air within the heel-in trench, suffered heavily from root rot. It appeared, therefore, that root-rot damage depended on whether or not the roots of the plants were exposed to temperatures below freezing, either at the time of digging or in improper storage.

As already noted, the storage studies undertaken at the Minnesota and Missouri nurseries were substantially the same as those undertaken in Iowa. Table 3 summarizes the results of the work in Minnesota.

TABLE 3.—*Root rot in black locust as related to storage method. Minnesota, 1939*

Materials	Indoor storage		Outdoor storage			Plants left in nursery rows for spring lifting
Nursery stock	Ricked and heeled-in in cellar	Ricked and heeled-in in cave	Heeled-in in sawdust	Heeled-in in shingle	Heeled-in in soil	
Root rot percentage[a]	35.0	35.0	100.0	100.0	35.0	0.0
Plants examined	10,500	1,200	600	600	600	1,000

[a] Percentage figures are average values for several tests.

The losses in outdoor storage in shingletow and sawdust were total. In all other forms of storage the amount of root rot proved curiously uniform,

varying from about 25 to 40 per cent, and averaging close to 35 per cent for all tests. This unusual uniformity was explained when it was found that at this nursery it required 12 days to lift the stock of black locust and on 5 of these days freezing or below freezing temperatures prevailed. Thus, approximately 42 per cent of the stock was exposed to freezing or below freezing temperature while it was being lifted. After lifting, the stock was transported to temporary storage pending grading and culling. This piling up of plants lifted on different days, and the grading and culling operations mixed the plants lifted under different temperature ranges. Unwittingly, plants from this mixture were selected the preceding fall for the storage tests. In such a mixture one would expect something less than 42 per cent of the roots to have been damaged by freezing and the recorded root decay of 35 per cent approached this figure in all forms of good storage where temperatures after storage seldom were below 40° F.

At the Missouri nursery appreciable amounts of root rot occurred only in shingletow and sand heel-in outdoor beds that were not mulched. The results obtained at the Minnesota and Missouri nurseries were consistent with the freezing relations deduced from the work in Iowa.

STORAGE STUDIES WITH OTHER DECIDUOUS SPECIES

The results of storage studies in Iowa with nursery species other than black locust are summarized in table 4.

Freezing injury of the roots apparently is the primary cause of root decay in storage of these species, as well as of black locust. In storage in shingletow outdoors the roots of all species except Siberian pea tree suffered heavily from root rot. In sand storage outdoors, a larger percentage of root rot occurred in the nonmulched than in the mulched beds. In soil, which was the densest heel-in medium used, the percentage of root rot was the least. In all indoor storage there was virtually no root rot, except for mulberry. Since this species also suffered heavily in soil storage, it is probable that at least part of the stock of mulberry was lifted while temperatures were below freezing.

It seems evident also that plants of different species vary in their tolerance of, or resistance to, low temperatures. Siberian pea tree, for example, escaped injury in all forms of storage where other species suffered much. Green ash is another, even hardier, species. The case of 77 per cent loss in shingletow storage is misleading. The molded condition of the roots was associated with severe injury to the roots by rodents. Osage orange, black walnut, and Russian mulberry are species that were found to be more susceptible to temperatures below freezing.

EXPERIMENTAL EXPOSURES OF SEEDLINGS TO SUB-FREEZING TEMPERATURES

The close relationship observed between exposure of the roots of plants to sub-freezing temperatures and the amount of root rot in storage called for a direct test of this factor. A preliminary test is described below.

TABLE 4.—*Percentage of root rot in nursery seedlings as related to storage method. Iowa, 1939*

Species	Indoor storage		Outdoor storage				
	Number of plants in test	Percentage of plants rotted	Number of plants in test	Percentage of plants rotted			
				Heeled-in in soil	Heeled-in in sand		Heeled-in in shingletow
					Mulched	Not mulched	
Osage orange	3,000	0.0	1,800	31[a] and 61	84.0	100.0	100.0
Black walnut	1,600	0.0	1,200	9.8	59.0	59.0	100.0
Russian mulberry	1,600	31.0	1,200	45.0	36.0	100.0	100.0
Wild plum	1,600	0.0	1,200	0.0	0.0	0.0	100.0
Siberian pea tree	3,000	0.0	1,800	0.0	0.0	0.0	0.0
Green ash	3,000	0.0	1,800	0.0	0.0	0.0	77.0
Bur oak	1,600	0.0	1,200	10.0	0.0	0.0	100.0
Gray dogwood	500	0.0	100	.....	0.0	0.0	.....
Riverbank grape	1,600	0.0	1,200	0.0	0.0	0.0	100.0
Prairie rose	600	0.0	100	.....	0.0	0.0	.....
Climbing bittersweet	400	1.3	100	.....	0.0	0.0	.....

[a] 31 per cent in the mulched and 61 per cent in the unmulched trenches. For the other species the difference was not appreciable.

On a February morning with the temperature at 17° F., 160 healthy black locust seedlings were taken from indoor storage and spread outdoors. At intervals of 20, 35, 60, 120, 165, 240, 300, and 360 minutes, 20 plants were returned to indoor storage. Each lot of 20 plants was divided into 2 loose bundles of 10 plants each. To 1 bundle 2 locust plants with rotted roots were added to serve as inoculum and a source of infection, while none was added to the other bundle. After exposure, all plants were stored indoors in moistened shingletow. Unexposed plants, with and without rotted roots, served as controls. The temperature at the beginning of the test and at the end of each interval of exposure noted above was 17, 17, 17, 18, 20, 23, 25, 25, 25 degrees F.

In this test all plants exposed to these temperatures, even for the short period of 20 minutes, suffered heavily from root rot in storage. The average loss for the 8 intervals of exposure was 92.5 per cent, and in no case was the loss less than 80 per cent. The plants to which rotted material was not added rotted just as completely as did those to which this material was added. All 20 plants not exposed to outdoor temperature remained healthy and entirely free from rot.

This preliminary work left little doubt that exposure of the roots to temperatures below freezing was the primary condition for the development of root rot in storage. There remained the problem of determining in more detail the temperature and duration of exposure that will effect permanent injury to the roots.

Freezing tests by exposing plants of various species to artificially regulated sub-freezing temperatures were undertaken during the storage season of 1939–40. The thermostatically regulated refrigerator of the Botany and Plant Pathology Department of Iowa State College was used for this purpose. The results of this work are summarized in figure 1.

Plants of the species noted in figure 1 were exposed to the ranges of temperature indicated by the arrows for periods of 5, 10, 20, 30, 45, 60, 120, 240, and 360 minutes. Plants of *Robinia,* for example, were exposed for periods of 5 to 360 minutes to the temperature ranges given, which are: 11–13° F., 15–16.5° F., and 24–25° F. The roots of all plants of *Robinia,* in all tests, exposed for 20 minutes or longer to any of the temperature ranges given, deteriorated in overwinter storage. Likewise, plants of the genera *Ulmus, Maclura,* and *Morus* required an exposure of only 20 minutes to a temperature range of 24–25° F. to effect permanent injury and predispose the plants to root decay in storage. Plants of *Juglans* were exposed only to the temperature range of 11–13° F. For this temperature the critical level of *Juglans* is 30 minutes, that is, plants exposed for 5, 10 and 20 minutes all fared well in overwinter storage, indicating that they were not permanently injured, while for exposures of 30 minutes or longer, the roots rotted in storage. Plants of *Quercus,* exposed only to the temperature range of 10–13° F., succumbed to root rot only after an exposure of at least 60 minutes. Plants of *Caragana,* not noted in the diagram, were exposed

to a temperature range of 12–17° F., but did not succumb to root rot until after an exposure of 120 minutes or longer. Plants of *Fraxinus* all fared well in storage, even after an exposure of 360 minutes or six hours to a temperature of 12–14° F.

With plants of *Prunus* the results were somewhat erratic. In 2 tests these plants succumbed to root rot after an exposure of only 20 minutes to temperatures of 15–16.5° and 21–23° F. In a third test the roots of the plants did not rot until after an exposure of 45 minutes or longer to a temperature range of 12–17.5° F. The type of root deterioration also differed

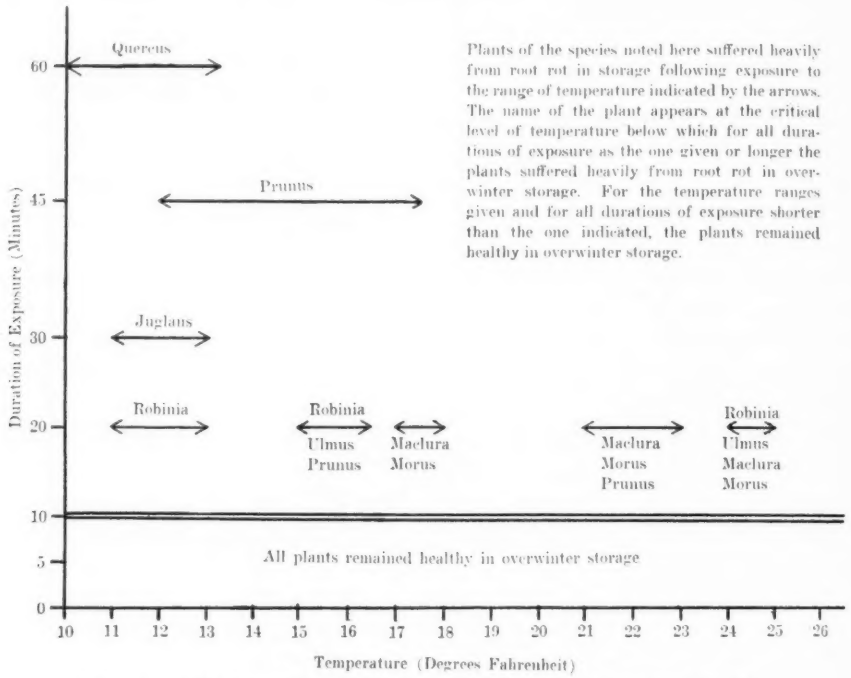
Quercus
Prunus
Juglans
Robinia
Robinia
Ulmus
Prunus
Maclura
Morus
Maclura
Morus
Prunus
Robinia
Ulmus
Maclura
Morus
All plants remained healthy in overwinter storage
Plants of the species noted here suffered heavily from root rot in storage following exposure to the range of temperature indicated by the arrows. The name of the plant appears at the critical level of temperature below which for all durations of exposure as the one given or longer the plants suffered heavily from root rot in overwinter storage. For the temperature ranges given and for all durations of exposure shorter than the one indicated, the plants remained healthy in overwinter storage.
Duration of Exposure (Minutes)
60
45
30
20
10
5
0
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Temperature (Degrees Fahrenheit)

FIG. 1. Critical temperatures of exposure for various nursery species.

in various tests. The type described for *Robinia* occurred sometimes, while in other tests the roots simply would dry and subsequently die with blue or green molds covering most of the plants.

THE ROLE OF FUNGI IN THE PRODUCTION OF ROOT ROT IN STORAGE

The tissues of rotted roots were almost invariably tinged with pink streaks, indicating the presence of *Fusarium* species. A Fusarium was always isolated from rotted roots. Other organisms isolated from rotted roots were species of *Alternaria, Mucor, Rhizopus, Penicillium,* a species of bacteria, and a fungus designated as isolate #6, which was not identified. Of these latter, *Alternaria* was the fungus most frequently isolated.

Each of the above-mentioned isolates was inoculated into 10 healthy seedlings of black locust through root incisions. The wound and inoculum were

wrapped with gauze and the plants were stored indoors in loose bundles of 10 plants protected with moist shingletow. Controls consisted of 10 additional healthy plants, similarly incised on the roots but without inoculum. After 60 days of storage the plants were examined and the following results were observed.

All 10 plants inoculated with *Fusarium* sp. showed some infection. The spread of infection from the point of inoculation in no case extended more than 2.5 cm. on either side of the wound. In 4 plants of the 10 the infection had penetrated to the side opposite from the wound, thus girdling the root. Some softening of the tissues in the immediate vicinity of the wound was noted but no progressive rotting up and down the root was evident. In plants first injured by low temperature the roots deteriorated in 2 or 3 weeks.

Six plants inoculated with *Alternaria* sp. showed some infections, while the other 4 remained entirely healthy. In the 6 plants showing infection the discoloration penetrated only to a depth of 3–4 mm. and extended for about 1.25 cm. on each side of the wound. No softening of the tissues or progressive rotting of the roots was evident in any of the plants.

The other organisms used for inoculation failed to infect any of the plants and remained perfectly healthy in storage. All control plants that were incised but not inoculated remained healthy.

These results indicate that species of *Fusarium* are probably weak parasites of black locust, unable to seriously parasitize healthy plants but able to infect and cause root decay to injured plants as by freezing or by severe wounding. It is not unreasonable, therefore, to ascribe a secondary role to these fungi in the rotting of the roots of deciduous nursery stock in overwinter storage.

DISCUSSION

The role of sub-freezing temperatures in predisposing nursery plants to root rot in overwinter storage has been shown in the preceding text. The storage work described for Iowa and Minnesota was repeated during the 1939–40 season with strikingly similar results. Cases of root rot in temporary field storage were traced repeatedly to unwitting exposures of the plants to temperatures below freezing as by leaving lifted plants overnight without adequate protection or leaving undercut stock in the nursery overnight, to shipping plants long distances in cold weather in plain unheated railway cars, or to faulty heel-in practices that permit mass movements of cold air within the heel-in trench as by using shingletow, sawdust, coarse sand, or insufficiently thick layers of soil in the heel-in trenches. A service memorandum issued to action agencies in the field warning of the danger of lifting stock in sub-freezing temperatures and of storing stock in improper storage resulted in elimination of practically all storage losses from root rot for 3 consecutive years where previously losses were experienced every year.

It has been noted earlier that losses of nursery stock at the Iowa and Minnesota nurseries have been repeatedly serious, while at the Missouri

nursery losses were seldom appreciable. Climate and local weather largely account for these differences. At the Missouri nursery only occasional severe temperatures occur before January and such spells of very cold weather are brief and transitory. At the Minnesota nursery, on the other hand, low temperatures may set in as early as the beginning of October. The nursery in Iowa is approximately midway, geographically, between the Minnesota and Missouri nurseries. Climatically it is much "nearer" the Minnesota nursery. At the Missouri nursery the plants for overwinter storage are lifted in December; at the Minnesota nursery, early in November; at the Iowa nursery, throughout November. A comparison of temperatures during periods of plant digging at these 3 nurseries showed that in Iowa freezing or sub-freezing temperatures prevailed in 8 of the 20 days required to lift the stock of black locust, or 40 per cent of the time; in Minnesota four days of the twelve, or 33 per cent of the time; while in Missouri the stock was lifted in 10 days in the month of December, and in every one of them mild temperatures prevailed. In view of these data, the weather factor explains why storage root rots in Iowa and Minnesota have been consistently serious, while in Missouri they have enjoyed considerable freedom from such troubles.

The conclusion is drawn that freezing injury has been the primary cause of major root deteriorations in storage of nursery seedlings in nurseries of the Upper Mississippi Valley. Fungi, of course, especially species of *Fusarium*, are essential to the destructive processes in root decay; but these activities must be classed as secondary in importance.

Differences in the relative resistance to low temperatures of the various species of plants used in these experiments are to be noted. Plants of black locust, Siberian elm, Osage orange, Russian mulberry, and wild plum apparently are the least resistant; while plants of Siberian pea tree and green ash, especially the latter, will withstand exposures to lower temperatures and for longer periods without serious injury.

SUMMARY AND RECOMMENDATIONS

Rather heavy losses of nursery seedling stock have been under observation at 3 nurseries in the Upper Mississippi Valley. It was found that freezing injury to the roots at the time of lifting, in transit, or in inadequate storage have been primarily responsible for root deteriorations in storage. The roots of several species of plants may be injured by short exposures to temperatures only a few degrees below freezing. Nursery practices that may lead to injury of nursery stock if carried out under sub-freezing temperatures are: lifting of stock, leaving undercut stock in loosened ground, transporting or other outdoor handling of unprotected stock, storing plants in outdoor heel-in beds without adequate protection to the roots from low temperatures (as when stored in sawdust, shingletow, coarse sand, or shallow soil covering), and storing plants indoors in poorly insulated structures that fail to keep temperatures above freezing in the coldest periods. In storage,

plants thus injured were found invaded by numerous fungi the most common of which were species of *Fusarium* and *Alternaria*. In wound inoculations *Fusarium* produced small local lesions on the roots of healthy plants but none of the organisms used caused extensive decay. Adherence to tentative recommendations formulated as a result of this work has reduced storage losses to a negligible figure in nurseries where there had previously been frequent losses.

As far as practicable deciduous nursery stock should be lifted and handled when the temperature is above freezing. This policy should apply especially to the following species: black locust, Siberian elm, Osage orange, black walnut, Russian mulberry, and tulip tree. If lifting of stock must be done at temperatures below freezing, it should be confined to species such as green ash or Siberian pea tree, which are more resistant to low temperature.

For outdoor storage in the northern nurseries, deep heeling-in is important. Loamy soils are preferred for this purpose to insure good root protection in the trench and good drainage. Avoid heeling-in stock in porous media as shingletow, sawdust, or coarse sand in regions where winters are severe. As a sanitation measure, heel-in sites should preferably be rotated from year to year.

Storage facilities such as packing sheds, root cellars, and caves, where temperature fluctuations are controlled within narrow limits above freezing (34° to 50° F.), and where a high relative humidity is maintained, appear to be safe for storing most deciduous nursery stock over winter.

Care should be exercised in lifting and handling nursery stock so as not to injure the roots any more than is absolutely necessary, since wounds open the way for infections. In grading large quantities of nursery stock all plants that show severe mechanical injuries to the roots or that show lesions, molds, and other types of root infection should be discarded.

When nursery stock, especially of the frost-susceptible species, is shipped in bales or crates it should not be placed outdoors in improvised shacks, in unheated nursery buildings, or in unheated railway buildings during freezing weather; and the planting operations themselves should not be done in such weather. Weather Bureau information can be used as a guide in shipping to avoid exposing nursery stock to sub-freezing temperatures while in transit.

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HISTOLOGICAL STUDIES OF INFECTIONS OF THE COTTON HYPOCOTYL BY GLOMERELLA GOSSYPII AND FUSARIUM MONILIFORME

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INTRODUCTION

Although several reports have been made concerning the pathogenicity of *Glomerella gossypii* (South.) Edg. and of *Fusarium moniliforme* Sheld. (1, 2, 3, 4, 5, 6, 7, 8) in cotton seedlings, apparently none has dealt with the problem from the histological point of view. It is the intent of this study to present histological evidence concerning the pathogenicity of the two organisms.

MATERIALS AND METHODS

Acid-delinted seeds of *Gossypium hirsutum* variety, "College No. 1," were immersed for 10 minutes in a 1–1000 aqueous solution of $HgCl_2$, washed with sterile water, and dried. They were then divided into 3 lots; one lot being plated directly on water agar to serve as a check, the second lot was thoroughly mixed in a spore and mycelium suspension of *Glomerella gossypii* derived from a single-spore isolate and plated on water agar, the third lot was mixed with a similar suspension of *Fusarium moniliforme* derived from a single-spore isolate and plated as above. The isolates from seedlings to provide the inoculum used in this experiment, had previously been shown by T. J. Rateliff to be virulent strains (unpublished data). Each lot consisted of 80 seeds plated in 20 Petri dishes containing 4 seeds each. The incubation temperature was 27° C. Fixations, in formal-acetic-alcohol, consisting of the seedlings from 2 plates from each group, were made daily for 8 days beginning on the second day following inoculation. Serial paraffin sections of a thickness of 20 μ, stained with Johansen's quadruple stain, were used.

RESULTS

Germination proceeded rapidly under the conditions of the experiment. As a result, the hypocotyls of most of the seedlings in all of the groups extended ½ to ¾ inch beyond the seed coat on the second day after inoculation. The hypocotyls of the seedlings of the check group continued to elongate until at the conclusion of the experiment (10 days), they had attained lengths of 3 to 5 inches. No lateral roots were formed and no evidence of infection was observed on any of the check seedlings.

Mycelium of *Glomerella gossypii* was found growing on the agar on the second day after plating. During the course of the experiment the hypocotyls infected with this fungus attained lengths of little more than 1 inch,

[1] The writer is indebted to George E. Thompson for the single-spore isolations, to T. J. Rateliff for assistance in the growth of the seedlings, and to O. K. Fletcher, Jr., for the photomicrographs.

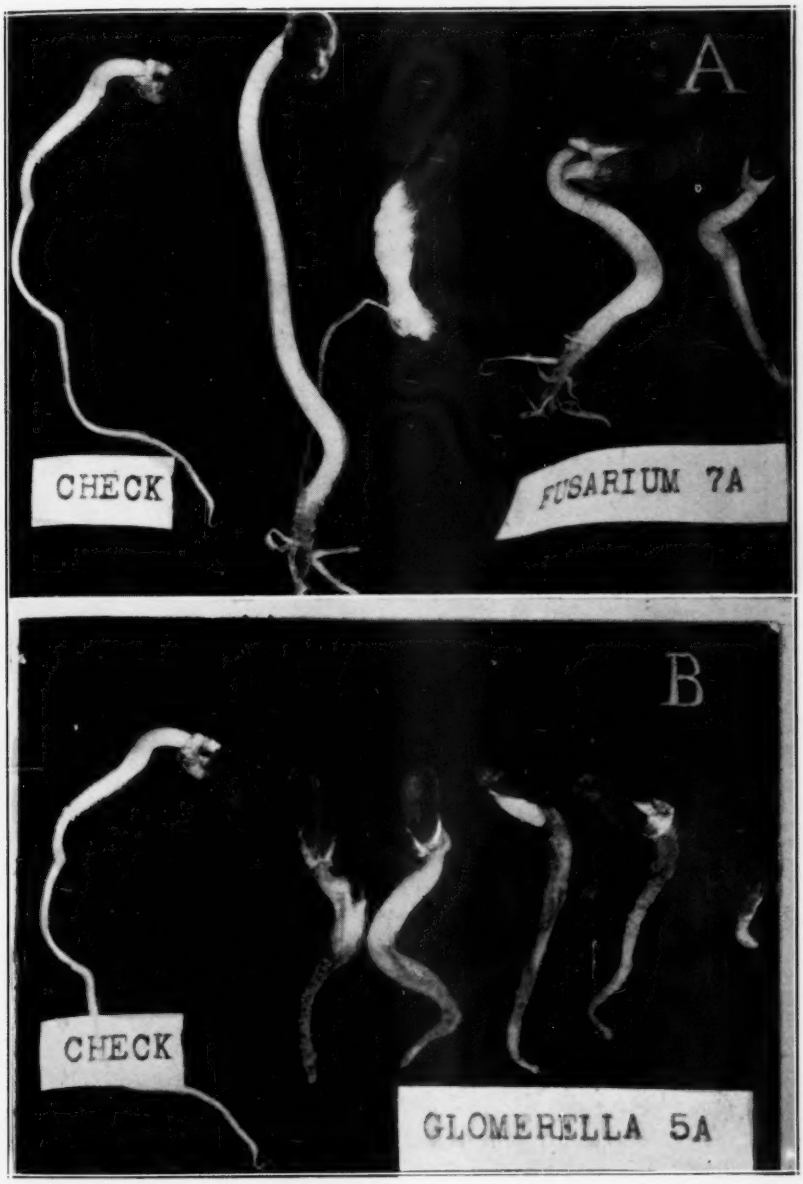

FIG. 1. Diseased cotton seedlings. A. Seedlings inoculated with *Fusarium moniliforme*, showing formation of lateral roots. B. Seedlings inoculated with *Glomerella gossypii*. ×1.25.

with an enlargement in diameter above the lesion and a constriction and apparent dying below that point (Fig. 1, B). No lateral roots were found on any of the hypocotyls infected by *G. gossypii*. Evidence of infection was found in cross sections of hypocotyls fixed on the fourth day after plating. At that time the mycelium was closely appressed to the surface of the hypo-

cotyl, was within root hairs and other epidermal cells, and also was within the parenchyma cells of the cortex, with certain individual strands extending nearly to the stele (Fig. 3, A, B). A flattening of the surface of the hypocotyl was noted at the apparent point of infection (Fig. 3, B). The mycelium spread rapidly in the horizontal plane within the cortex and into the stele. Strands of mycelium were observed wrapped around nuclei.

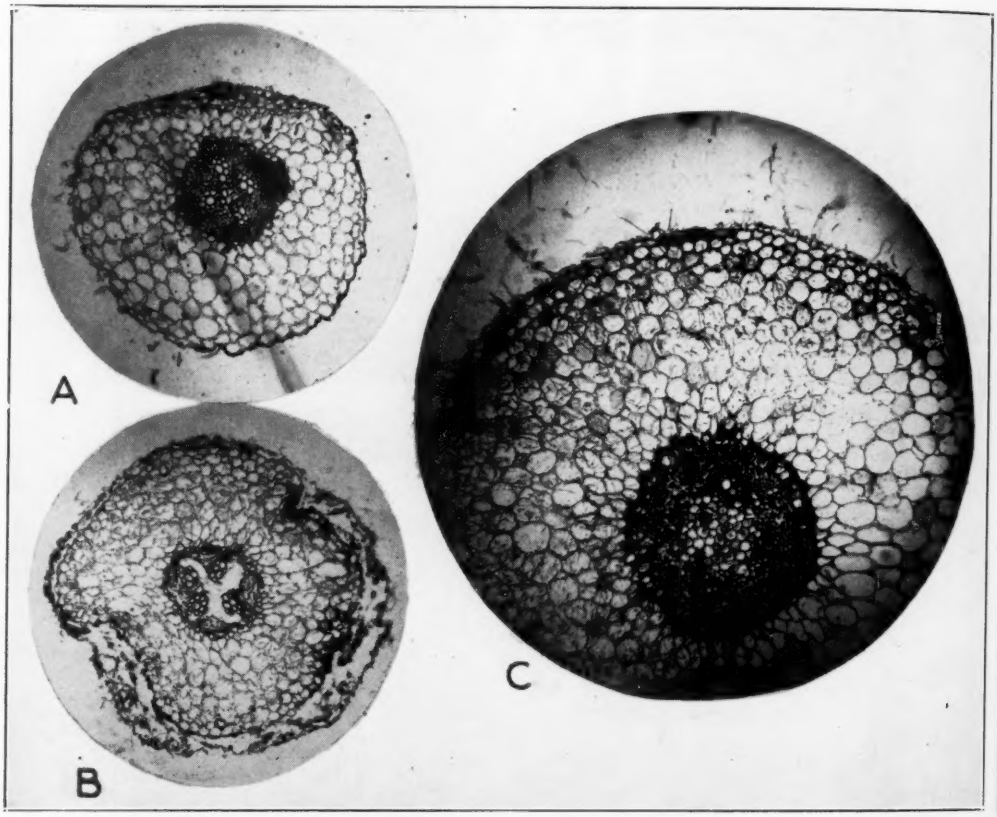

FIG. 2. Cross sections through cotton hypocotyls showing stages in the advance of the mycelium of *Fusarium moniliforme* within the hypocotyl. A. Early stage showing flattening of the side and the initiation of a lateral root near the point of infection. B. Later stage showing mycelium throughout cortex and within the stele and forming halo surrounding the hypocotyl. C. Intermediate stage showing localized mycelium penetrating as far as the stele. ×50.

Early stages of infection by *Glomerella gossypii* resulted in little or no apparent injury to individual cortical cells, (Fig. 3, A, B), while later stages show an apparent shrinking and indications of necrosis (Fig. 4, A, B; 5, B). In certain cases the infection remained localized, affecting only one side (Fig. 5, A), while in others the entire cross section of the hypocotyl was infected (Figs. 4, B; 5, B). Mycelium was found in the stele after considerable damage had been done to the cells of the cortex (Fig 5, B). All of the later stages revealed a halo of rather heavy external mycelium surrounding the hypocotyl on which acervuli were developing (Figs. 4, A, B; 5, A, B).

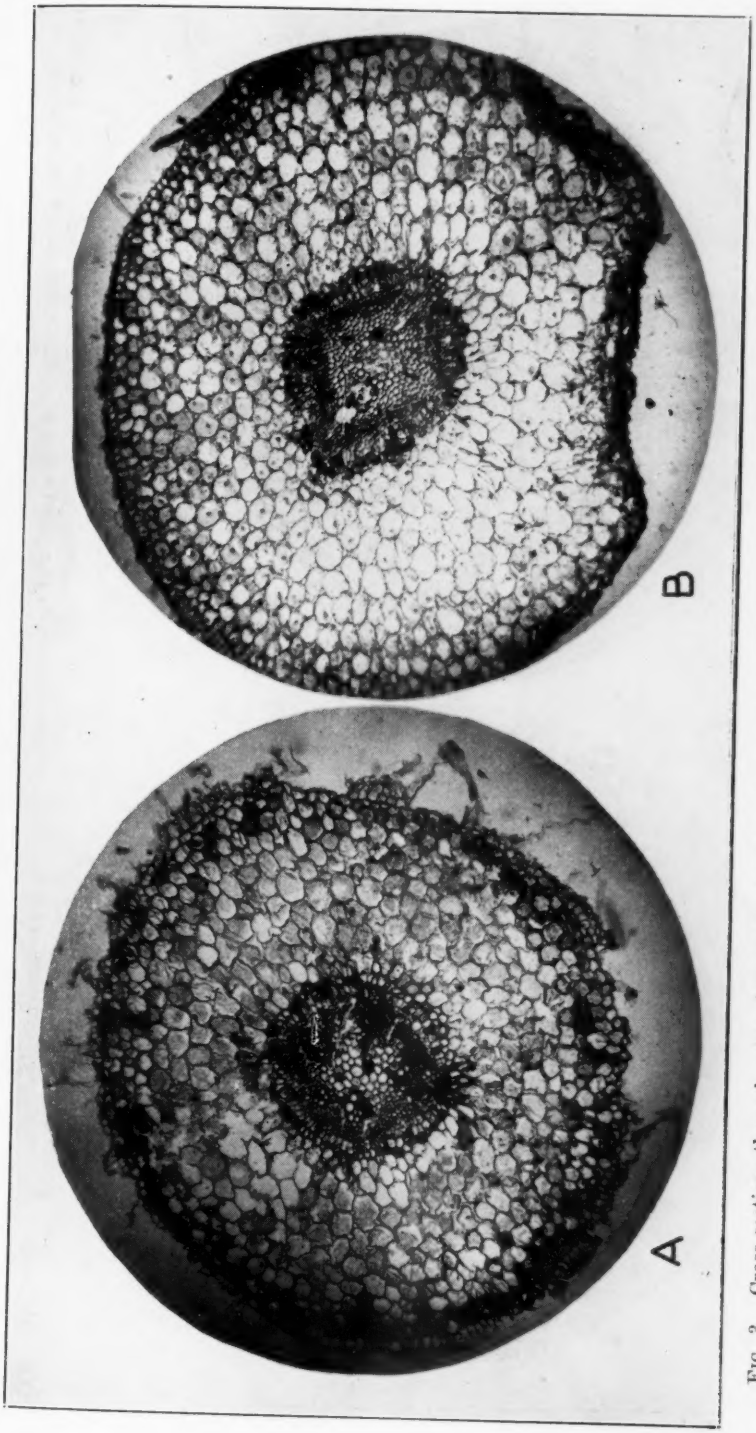

FIG. 3. Cross sections through cotton hypocotyls showing stages of penetration by *Glomerella gossypii*. A. Small amount of mycelium in upper, lower and left sides of section of hypocotyl. B. Flattening of side of hypocotyl at point of infection, individual strands of mycelium penetrating most of distance to the stele. ×50.

FIG. 4. Cross sections of hypocotyls infected by *Glomerella gossypii*. A. Heavy infection within cortical cells and acervuli upon the surface. B. Later stage, showing some shrinkage of cells, small amounts of acervuli and penetration of cortex and stele by the mycelium. ×50.

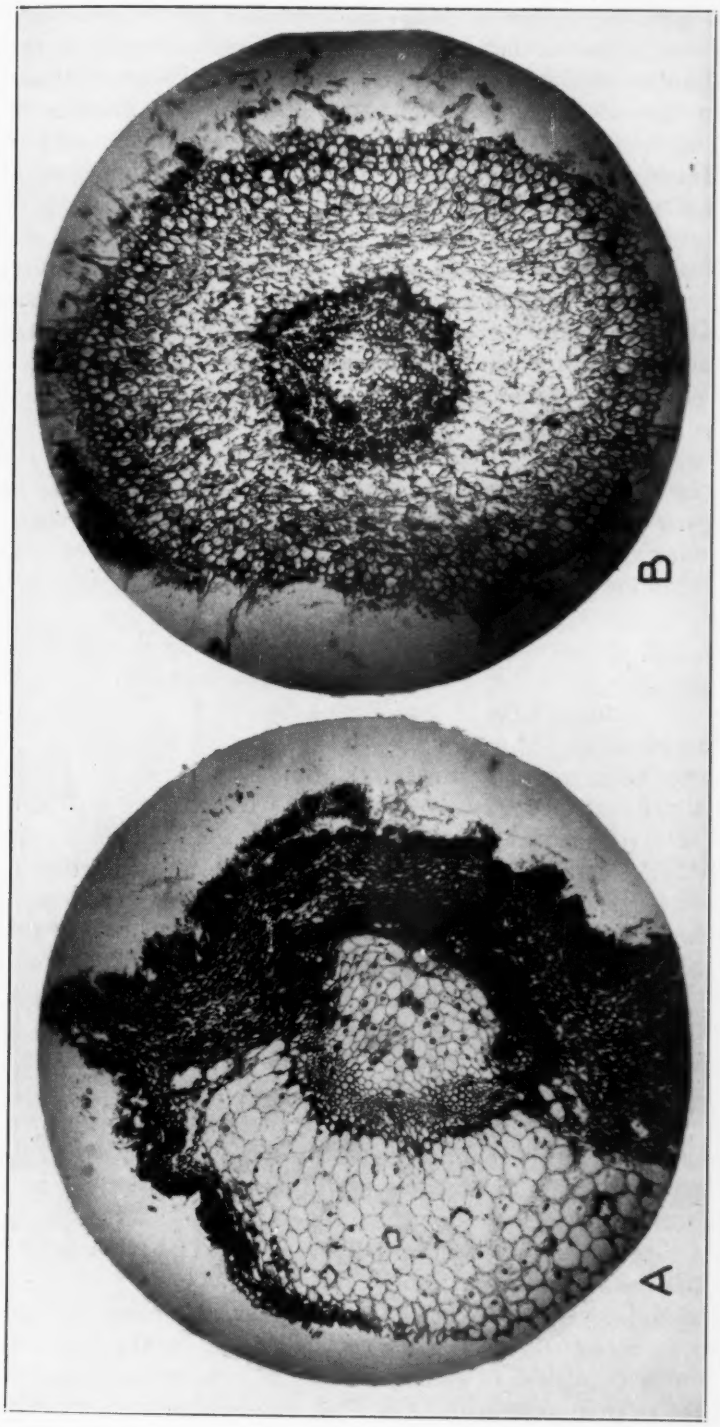

FIG. 5. Cross sections of cotton hypocotyls in late stages of infection by *Glomerella gossypii*. A. One side heavily infected, other side clear of infection. B. General penetration of mycelium throughout cortex and stele, with acervuli upon the surface and showing evident necrosis. ×50.

Infection of the cotton seedlings by *Fusarium moniliforme* followed much the same course as that described for *Glomerella gossypii*, as regards the time of appearance upon the agar, the entrance, and growth of the organism within the cells of the host (Fig. 2, A, B, C). The presence of the mycelium of *F. moniliforme* resulted in a similar effect on the cells of the cortex and stele, in a similar enlargement of diameter of the hypocotyl above the region of infection (Fig. 1, A), and in the flattening of the side of the hypocotyl at the point of infection (Fig. 2, A).

There were, however, several points of difference between the effects of the two organisms. The hypocotyls infected by *Fusarium moniliforme* in some cases attained lengths as great as the checks (5 inches), had less external mycelium, and were generally characterized by the appearance of a whorl of lateral roots at the region of infection (Figs. 1, A; 2, A).

DISCUSSION

Since the mycelium of both *Glomerella gossypii* and *Fusarium moniliforme* apparently invaded the hypocotyl of cotton seedlings through root hairs and other epidermal cells, penetrated living cells of the cortex and stele and resulted in the death of these cells, it would appear that both organisms are primary intracellular parasites.

Infection by *Glomerella gossypii* has been reported by several writers as causing more damage to the cotton plant than does infection by *Fusarium moniliforme*. Arndt (1) found that *F. moniliforme* "will infect seedlings and produce stunting, but rarely death or damping-off of seedlings. Some parallel experiments with the anthracnose fungus under similar conditions showed 100 per cent killing, whereas there was 100 per cent survival with *F. moniliforme* though many of the seedlings were smaller than the disease-free controls." Arndt and Christie (2) reported that infection by *F. moniliforme* reduced the germination of cotton seed by 10 to 15 per cent, tended to increase the number of lesions on the hypocotyls, and to produce very irregular plants, but not to result in typical damping-off. *G. gossypii* caused typical damping-off in their experiments, killed 72 per cent of the plants and caused the surviving plants to be small and diseased.

Weindling *et al.* (7) noted that "*F. moniliforme* does not appear to be a primary pathogen of the damping-off of cotton seedlings," and in discussing their survey, found it "difficult at the present time to evaluate the high percentage of *F. moniliforme* obtained." These writers further stated that *G. gossypii* had been highly destructive in all cases. Woodroof (8) reported *F. moniliforme* as being the cause of stunting of cotton plants, but she did not believe that the organism entered living cells. The latter point is in variance with the findings of this study.

Ray and McLaughlin (5) reported both organisms as being pathogens of the cotton seedlings, *Glomerella gossypii* resulting in the more serious injury. Under conditions of excessive moisture these writers noted an increase in the relative pathogenicity of *Fusarium moniliforme* and with de-

creased moisture, lower temperature and increased alkalinity a relative increase in the pathogenicity of *G. gossypii*.

The differences in response of the cotton seedlings to infection by the two organisms cannot be explained on the basis of entrance and course of the mycelium within the cortex and stele of the hypocotyl. It may be inferred, although it is not demonstrated in this study, that differences in the spread of the organisms in the vertical plane might account for the differences in the effect on the host. Seedlings infected by *Fusarium moniliforme*, grown in Petri plates, attained in many cases normal or nearly normal lengths, while those infected by *G. gossypii* failed to elongate.

In the case of those seedlings infected by *Fusarium moniliforme* there was an appreciable tendency toward the initiation of lateral roots at the margin of the infected area, a condition totally absent, in this experiment, in the anthracnose-infected seedlings. A possible inference is that the initiation of the whorl of lateral roots is stimulated by some substance produced by the mycelium of *F. moniliforme* as it grows within the cells of the cortex.

SUMMARY

Infections by *Glomerella gossypii* or by *Fusarium moniliforme* in cotton seedlings, in Petri plates, behaved similarly as regards penetration and course of growth within the host. Both are evidently intracellular parasites, which eventually induce death of the host cells. The greater tendency for survival following infection by *F. moniliforme* may possibly be accounted for by the stimulation of the production of lateral roots.

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THE SENSITIVITY OF PLANT VIRUSES TO CERTAIN INACTIVATORS[1]

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INTRODUCTION

In previous publications from this laboratory the inactivating effects of certain chemical substances upon plant viruses have been investigated, (1, 9, 10, 14). Particular interest has developed in the effect of several so-called "non-toxic" inactivators. A "non-toxic" inactivator is here meant to include a substance not detrimental to most forms of life, but one that does inactivate plant viruses. These include certain plant extracts, milk, trypsin, normal blood serum, and other complex substances of biological origin.

Most investigations of "non-toxic" inactivators have been concerned with but a single virus, namely, the ordinary tobacco-mosaic virus. Since other plant viruses have proved much more sensitive to toxic chemicals than has tobacco-mosaic virus (1), it appeared that some significant information might be secured by comparing the action of "non-toxic" substances upon these different viruses. Although it may be assumed that the nature of the reactions of viruses to toxic substances differs from that of their response to "non-toxic" ones, the magnitude of this difference is not at all clear. The present investigation was undertaken with the hope that detailed data on the effects of "non-toxic" inactivators on several viruses might provide evidence of the nature of this type of inactivation. The results obtained show that viruses differ in their sensitivity to the "non-toxic" inactivators used, as they also have been shown by Allington (1) and others to differ in their sensitivity to toxic chemicals.

LITERATURE REVIEW

The first description of the ability of a supposedly innocuous substance to inactivate the tobacco-mosaic virus was by Duggar and Armstrong (6) for the juice of *Phytolacca decandra*. Numerous other substances have since been found to inactivate this virus. These include certain other plant juices (7), trypsin (12), normal blood serum (11), juices of certain insects (3), milk (5), casein, albumen, peptone (4), growth products of several species of microorganisms (10), and possibly tannic acid (13). While most of these have been investigated in their relation only to the tobacco-mosaic virus, Stanley (12) reported that trypsin inactivated the viruses of cucumber mosaic, severe etch, and tobacco ring spot. Chester (5) reported that normal blood serum inactivated the viruses of tobacco ring spot and cucumber mosaic, and Black (3) found that extracts of clover leaf-hoppers inac-

[1] Supported in part by allotments from the University of Wisconsin Research Fund. The author wishes to express his thanks to Dr. James Johnson for helpful advice and criticism during the course of the work and preparation of the manuscript.

tivated the viruses of potato yellow dwarf, potato X, turnip mosaic, tobacco necrosis, and tobacco ring spot. Johnson (9) reported that both milk and *Aerobacter aerogenes* growth product inactivated a number of viruses, including those of cucumber mosaic, potato veinbanding, and potato ring spot.

While there has been a lack of agreement as to the nature of the effect of these inactivators, certain features of their action are characteristic for the group, and have been observed repeatedly. For nearly all the substances investigated, inactivation has been observed immediately on mixing with virus (2, 3, 5, 7, 9, 12). Reactivation of the virus following removal or destruction of the inactivator has been reported also for all the "non-toxic" inactivators (5, 9, 12), except Phytolacca extract.

The immediate effect of these inactivators and the temporary nature of that effect have led to the belief that the virus itself is not affected, but rather that the inoculated host is rendered less susceptible (5, 12). The principal proof advanced by Stanley (12) in favor of this view is based on a test in which a series of virus dilutions are mixed with constant amounts of inactivator, and the percentage inactivation determined by inoculation to a host yielding local lesions. A greater percentage reduction of local lesions with the more dilute extracts is accepted as indicating an effect on the virus, while a similar percentage reduction at all dilutions is accepted as indicating an effect on the host. Results of this test, particularly with trypsin and blood serum, have been interpreted differently by different investigators (4, 5, 12, 15).

Evidence also has been presented indicating that certain inactivators affect the virus. Hills and Vinson (8) showed that the rate of diffusion of tobacco-mosaic virus particles was slowed by the presence of trypsin, and concluded that the virus and the trypsin combined. Johnson (9) has shown that for many toxic chemicals a period of contact with the virus is necessary for maximum inactivation. This method of proof cannot be used with the "non-toxic" inactivators, which exert their full effect immediately on mixing with virus. It was hoped that the present investigation might yield additional evidence as to whether the host or the virus was affected, or that it might suggest methods of obtaining such evidence.

METHODS AND MATERIALS

The following 5 "non-toxic" substances were selected as representative of this class of inactivators: *Phytolacca decandra* L. extract (stored under toluene at 4° C.), trypsin (Difco, standardized), fresh cow's milk, bovine serum (stored frozen), and *Aspergillus niger* van Tiegh. growth product (the liquid from a two weeks broth culture evaporated to 1/10 original volume, stored frozen). The viruses used were those of tobacco mosaic (*tobacco virus 1*), tobacco ring spot, cucumber mosaic (*cucumber virus 1*), potato ring spot, and bean mosaic. Young Havana Seed No. 38 tobacco plants were used in tests for the first 4 viruses, and Wisconsin Refugee Stringless bean seedlings for the bean-mosaic virus.

While the local-lesion method is rapid and relatively accurate for determining inactivation, it could not be used conveniently with several of the viruses. Therefore, in order to make the results as comparable as possible, inactivation of all viruses was determined by inoculating 5 young healthy plants in each test, and recording the number that became infected as a measure of the degree of inactivation. Five such tests, involving a total of 25 plants for each concentration of inactivator, were made for each mixture. It is recognized that this method determines only the concentration at which inactivation is complete, or very nearly so.

To provide inoculum, young, recently infected plants were ground up, the juice filtered through cheese cloth and then diluted. In preliminary tests it was found necessary to dilute the tobacco-mosaic virus more than the other viruses in order to obtain a demonstrable degree of inactivation. Similarly some of the inactivators were less potent; this necessitated using greater dilutions of all the viruses. All inoculations were made within 2 or 3 minutes after mixing the virus and the inactivator.

EXPERIMENTAL RESULTS

Relative Sensitivity of the Viruses to Inactivation

As illustrative of the type of results obtained, the detailed data on 2 viruses and 2 inactivators are presented in table 1. The results obtained on the inactivation of all 5 viruses with the 5 inactivators are summarized in table 2. From the data in this table the 5 viruses may be arranged in the order of their sensitivity to the inactivators as a group. Thus, bean-mosaic virus usually required the lowest concentration for complete inactivation,

TABLE 1.—*Representative data showing the amount of inactivation induced by various concentrations of two inactivators on two viruses diluted to 1:1000*

Inactivator	Virus of	Percentage concentration of inactivators and number of plants infected out of 25 inoculated											
		80	70	60	50	40	30	25	20	15	10	5	0
Bovine serum	Tobacco ring spot	.....	.....	.....	.....	.....	0	0	1	3	8	16	25
	Cucumber mosaic	.....	.....	.....	.....	.....	0	3	5	6	9	14	25
Aspergillus growth product	Tobacco ring spot	0	3	2	3	9	.....	.....	13	.....	.....	.....	25
	Cucumber mosaic	.....	.....	.....	.....	0	3	.....	9	11	13	18	25

followed by tobacco-ring-spot virus, cucumber-mosaic virus, potato-ring-spot virus, and tobacco-mosaic virus, in that order. This same order of sensitivity for the latter 4 viruses also was found by Allington (1) when toxic chemicals were used. He found that the cucumber mosaic and potato-ring-spot viruses did not differ greatly in their sensitivity to the toxic chemicals. In the present investigation, however, using "non-toxic" inactivators, the

potato-ring-spot virus was definitely more tolerant than the cucumber mosaic virus.

There were, however, some marked variations in the order of sensitivity of the viruses to certain inactivators. The tobacco-ring-spot virus, for example, is inactivated by a lower concentration of bovine serum than is the cucumber-mosaic virus. The latter, however, is inactivated by a lower concentration of *Aspergillus* growth product than is the tobacco-ring-spot virus (Table 1). Similarly, a higher concentration of Phytolacca extract is necessary to inactivate the bean-mosaic virus than is required for the tobacco-

TABLE 2.—*Summary showing concentrations of 5 inactivators required to inactivate 5 different viruses. (Each figure based on 5 tests of 5 plants each)*

Inactivator	Concentration[a]	Viruses, at dilutions shown, and percentage concentration of inactivator required				
		Bean mosaic 1:10	Tobacco ring spot 1:100	Cucumber mosaic 1:100	Potato ring spot 1:100	Tobacco mosaic 1:1000
Phytolacca juice	A	0.25	0.2	1.25	3.0	5.0
	B	0.125	0.1	1.0	2.5	4.0
Milk	A	7.5	25.0	20.0	.....	.....
	B	5.0	20.0	15.0	99.0[b]	99.0[b]
Trypsin	A	0.25	1.0	3.0	3.5	.....
	B	0.125	0.5	2.5	3.0	3.5[b]
		Bean mosaic 1:10	Tobacco ring spot 1:1000	Cucumber mosaic 1:1000	Potato ring spot 1:1000	Tobacco mosaic 1:10,000
Bovine serum	A	10	25	30	.....	.....
	B	5	20	25	99[b]	99[c]
Aspergillus growth product	A	.....	80	40	.....	.....
	B	90[b]	70	30	99[c]	99[c]

[a] A—Lowest percentage concentration resulting in complete inactivation.
B—Highest percentage concentration not resulting in complete inactivation.
[b] One to 5 plants infected out of 25 inoculated.
[c] Ten to 20 plants infected out of 25 inoculated.

ring-spot virus; whereas considerably higher concentrations of the other inactivators are required for the tobacco-ring-spot virus. The actual amount of virus present was undoubtedly different in the original preparations of different viruses. The relative concentrations of the viruses, however, were constant in the trials with the different inactivators. The results indicate, therefore, that properties inherent to the virus affect its sensitivity to "non-toxic" inactivators, as has been demonstrated previously for toxic chemicals (1).

The Effect of Virus Concentration on Inactivation

The results in table 2 seemed to indicate that the concentrations of the viruses influenced to some degree the concentration of inactivator required. Tobacco-mosaic and potato-ring-spot viruses, which required the highest con-

centrations of the inactivators, exist in high concentrations in infected tobacco plants, whereas the viruses of cucumber mosaic and tobacco ring spot exist in much lower concentrations. In comparing the effectiveness of a single inactivator on different viruses the relative infectivities of the virus preparations should be considered. The concentrations of the viruses in the extracts were compared, therefore, by means of dilution tests. Five plants were inoculated in each of 5 trials with a given dilution (Table 3). It is difficult to compare the viruses because of the differences in the shape of the dilution curves. Infectivity of the extracts of tobacco-ring-spot virus dropped sharply on dilution, while those of cucumber-mosaic virus, over

TABLE 3.—*Relative infectivities of 5 viruses as determined by their tolerance to dilution, with the same method of inoculation used in all trials*

Virus of	Dilution and number of plants infected out of 25 inoculated														
	1:30	$1:10^2$	$1:3\times10^2$	$1:10^3$	$1:3\times10^3$	$1:10^4$	$1:3\times10^4$	$1:10^5$	$1:3\times10^5$	$1:10^6$	$1:3\times10^6$	$1:10^7$	$1:3\times10^7$	$1:10^8$	$1:3\times10^8$
Bean mosaic	24	18	5	4	2	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Tobacco ring spot	.....	.....	.....	25	21	10	0	0	.....	.....	.....	.....	.....	.....	.....
Cucumber mosaic	.....	.....	.....	.....	9	9	5	2	1	0	.....	.....	.....	.....	.....
Potato ring spot	.....	.....	.....	.....	.....	.....	.....	.....	15	6	5	4	0	0	.....
Tobacco mosaic	.....	.....	.....	.....	.....	.....	.....	.....	.....	22	14	1	1	0	0

much the same range, lost infectivity much more gradually on dilution. Comparisons are thus only approximate. If the infectivity of the bean-mosaic virus extracts is taken as 1 the relative infectivities of the virus extracts of tobacco ring spot, cucumber mosaic, potato ring spot, and tobacco mosaic are about 30, 100, 10,000, and 30,000, respectively.

The virus extracts of bean mosaic, tobacco ring spot, and cucumber mosaic, as used in inactivation trials, were nearly equal in infectivity. In general, the lowest concentrations of inactivator were required for the bean-mosaic-virus extracts, which were also the least infective. Higher percentages of inactivator were usually required for the more infective virus extracts of tobacco ring spot and cucumber mosaic. Less inactivator was required, however, for the viruses of potato ring spot and tobacco mosaic than would be expected on the basis of their greater infectivities. For example, 3.0 per cent trypsin is required to inactivate the cucumber-mosaic virus at a dilution of 1:100, and 3.5 per cent trypsin is required for potato-ring-spot virus at the same dilution, yet the infectivity of the latter virus was about 100 times as great as the former. When different dilutions of viruses are mixed with an inactivator some of this effect might be due to the higher concentration of extraneous materials in the least dilute extract interfering with the activity of the inactivator. This should not be im-

portant, however, in the case of extracts tested at the same dilution. Either the potato-ring-spot and tobacco-mosaic viruses are more susceptible to all the inactivators, or, since they were used at higher concentrations than the others, their concentration may have been responsible for their inactivation by relatively low percentages of inactivators.

As a means of determining the effect of virus concentration on inactivation 3 dilutions of tobacco-mosaic virus were mixed with 3 dilutions of inactivator, keeping the ratio of virus to inactivator the same at each dilution. These mixtures were inoculated to the *Nicotiana tabacum* × *N. glutinosa* hybrid leaves, yielding local lesions, in a 3 × 3 Latin square arrangement, with the mixture on one-half of a leaf, and the corresponding dilution of virus alone on the other half. The results with 4 inactivators are presented in table 4. With this virus the inactivation percentage decreased as the concentration of the mixture became lower. It seems likely, therefore, that the greater infectivities of the extracts of tobacco-mosaic

TABLE 4.—*The relation of the concentration of mixtures of the tobacco-mosaic virus and inactivators to the amount of inactivation resulting*

Mixture			Total number of lesions on test plants produced by		
Virus concentration	Inactivator and concentration		Virus plus inactivator	Control (virus only)	Percentage reduction due to inactivator
		Per cent			
1: 500	Trypsin	0.4	27	1139	97.6
1: 2,500	"	0.08	55	178	68.7
1: 12,500	"	0.016	48	95	49.5
1: 500	Milk	8	14	1020	98.6
1: 2,500	"	1.6	25	501	95.1
1: 12,500	"	0.32	27	96	71.9
1: 500	Phyt. juice	0.15	44	1920	97.7
1: 2,500	" "	0.03	62	821	92.5
1: 12,500	" "	0.006	73	219	67.7
1: 500	Blood serum	8	25	1467	98.3
1: 2,500	" "	1.6	88	294	70.1
1: 12,500	" "	0.32	71	72	1.4

and potato-ring-spot viruses resulted in their being inactivated by proportionately lower concentrations of inactivators than the more dilute viruses. The reactivating effect of dilution on mixtures of some of these inactivators has been reported (5, 12).

An interesting, and probably significant, observation on the effect of dilution is the difference in degree to which it affects the different inactivators. The inactivation with milk dropped only from 98 to 72 per cent on dilution. On the other hand, diluting bovine serum mixtures to the same extent decreased the inactivation from 98 per cent to practically no inactivation. These results seem best explained on the basis of a difference in tenacity of combination between virus and inactivator.

DISCUSSION

An accurate comparison of the sensitivities of viruses to a particular inactivator probably would require the use of purified virus, purified inactivator, and virus preparations with the same concentration of infective particles. Since these requirements could not be met in the present investigation, an attempt was made to secure relative data by comparing the sensitivities of several viruses to several inactivators. It is, for example, of little significance to determine that the tobacco-ring-spot virus is inactivated by a lower concentration of bovine serum than the cucumber-mosaic virus, since the actual amount of virus being inactivated in each case is not known. If in addition, however, the same dilution of cucumber-mosaic virus is inactivated by a lower concentration of *Aspergillus* growth product than the tobacco-ring-spot virus, then a difference between the sensitivities of these two viruses may be assumed. Several such differences between the viruses of bean mosaic, tobacco ring spot, and cucumber mosaic were demonstrated. These differences seem to be further evidence that the effect of "non-toxic" substances is on the virus itself, and not on the host.

The results of diluting mixtures of inactivator and virus also throw some doubt on the validity of dilution tests used to determine whether a substance affects the virus or the host. This test assumes that, if the virus is affected, a definite amount of inactivator will act upon a definite amount of virus regardless of the relative concentrations of the two. On the basis of the results presented in table 4, however, mixtures of a high virus concentration and a definite amount of inactivator may be expected to show a larger proportion of the virus inactivated than of mixtures with a lower virus concentration. This is essentially the result obtained with trypsin in dilution tests involving mixing constant concentrations of inactivator with different dilutions of virus, and that has been interpreted as being due to an effect of trypsin on the host. When the effect of dilution on an inactivator is relatively large, results would be obtained indicating that the effect of the inactivator is on the host. It may be significant in this connection that there is a relatively great drop in percentage of inactivation when mixtures of trypsin or blood serum are diluted. Both these substances have been described as examples of "inhibitors" that do not act on the virus.

The results obtained offer no evidence contradictory to the idea, as expressed by Thornberry (13), that this type of inactivation may be due to a reversible interaction between inactivator particles and virus particles. Earlier work has shown that, except for Phytolacca extracts, inactivation is reversed when the inactivator is removed or destroyed. In connection with the present investigation it has been found that virus mixtures with Phytolacca extract may also be reactivated by the proper technique.[2] All the

[2] Virus extracts of potato ring spot, cucumber mosaic, tobacco ring spot, and tobacco mosaic were each mixed, as in table 2, with the lowest percentage of Phytolacca extract necessary for complete inactivation. Talc was then added at the rate of 2 g. per 50 cc. of mixture, shaken thoroughly, then allowed to settle. The decanted liquid yielded 100

"non-toxic" inactivators used, therefore, seem to behave similarly in this respect.

While the nature of the effect of an inactivator on a virus is not known, some evidence suggests that a reversible adsorption may be concerned. This conception need not be entirely incompatible with the evidence pertaining to the reactivation of virus by ultra filtration, ultra centrifugation, or diffusion techniques. Such a possibility should, at least, be considered before attributing the principal effect of "non-toxic" inactivators to a reduction of host susceptibility.

SUMMARY

The sensitivity of viruses to inactivation by trypsin, milk, extract of *Phytolacca decandra*, bovine serum, and *Aspergillus niger* growth product was determined. The viruses used were those of tobacco mosaic, potato ring spot, cucumber mosaic, tobacco ring spot, and bean mosaic. In general, the sensitivity of the viruses to the inactivators increased in the order named above. The viruses of bean mosaic, tobacco ring spot, and cucumber mosaic, however, exhibited specific responses to the extent that the order of sensitivity to certain inactivators was reversed. The virus extracts of tobacco mosaic and potato ring spot were much more concentrated than the other virus extracts, but required only moderately higher concentrations of inactivators. The percentage of tobacco-mosaic virus inactivated was greatest when the mixture with inactivator was most concentrated, least when the mixture was most dilute. As a whole, the results supported the view that the effect of the "non-toxic" inactivators is on the virus and not on the host.

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per cent infection in each case, whereas mixtures not treated with talc produced no infection. Using the local-lesion method with the tobacco-mosaic virus it was noted that the reactivation was not necessarily always complete.

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ORGANIC MATERIALS IN PRE-HARVEST SPRAYS FOR CHERRIES

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The standard practice of applying sulphur sprays and dusts for controlling the brown rot, *Sclerotinia fructicola,* Wint. Rhem., and gray mold, *Botrytis cinerea* Pers., of sweet cherries has not been entirely effective. Irrespective of control, the various sulphur treatments have one serious defect: They all leave noticeable deposits of spray residue. And spray residues of any nature are highly objectionable to fruit consumers.

Since 1938, the writers have been trying to find some material, other than sulphur, that could control brown rot of cherries, without visible residue on the fruit. They reported[2] promising results from the use of an extremely dilute red copper oxide spray, at $\frac{1}{4}$ lb. in 100 gal. of water, plus a spreader. This spray, however, causes copper injury which necessitates an after-harvest lime spray to prevent serious leaf drop. To overcome the undesirable properties of the sulphur and copper fungicides, recent investigations were conducted to test certain of the most promising organic materials.

MATERIALS TESTED

Three of the new organic materials, recently shown to be toxic to fungi at low concentrations, were tested as pre-harvest applications on sweet cherries. They were ferric-dimethyl-dithio-carbamate (Fermate), tetra-methyl-thiuram-disulphide (Jap Beetle Spray), and tetrachloro-para-benzoquinone (Spergon).[3] Micronized sulphur was used as the standard treatment. S. E. C. Oil (self-emulsifiable cottonseed oil) was employed as a spreader and sticker in comparative tests to determine whether it enhanced control of the very dilute fungicide applications that were necessarily employed to avoid residues.

DISEASE CONTROL

Spore Germination Experiments

Methods. In conjunction with the field control tests, spore germination tests[4] were made to determine the relative toxicity of the Jap Beetle Spray and Fermate materials in comparison with sulphur and red copper oxide. The glass slides used in these tests were coated with cellulose nitrate. The slides were exposed to a given spray for 15-, 60-, and 120-second intervals.[5]

[1] Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 532, November 7, 1942.

[2] Parrott, P. J. New York Agr. Exp. Sta. Ann. Rpt. 1938: 24. 1939.

[3] Fermate and Jap Beetle Spray were supplied by duPont de Nemours Co. Spergon was furnished by the U. S. Rubber Co. Red copper oxide and S. E. C. Cottonseed Oil were from Röhm & Haas.

[4] Horsfall, J. G., J. W. Heuberger, E. G. Sharvelle, and J. M. Hamilton. A design for laboratory assay of fungicides. Phytopath. 30: 545–563. 1940.

[5] Hamilton, J. M., and G. L. Mack. A new vertical laboratory sprayer (in preparation).

One half of the slides for each treatment were subjected to washing by passing the slides 30 times through distilled water after the spray had thoroughly dried.[6] The other half were left unwashed. Each of the 4 fungicides was used alone and with S. E. C. Oil, 1–1600. Three uniform drops of a spore suspension of *Sclerotinia fructicola*, adjusted to a concentration of 60 spores per sq. mm. in a Fuchs-Rosenthahl counting cell, were placed on each slide. After incubation in moist chambers at a temperature favorable for germination, 100 spores were counted from each of 3 drops and averaged for the percentage of spore inhibition.

TABLE 1.—*Comparative toxicity of organic and inorganic fungicides to spores of Sclerotinia fructicola on coated glass slides*[a]

Treatment	Grams per liter	Seconds exposed to spray	Ave. percentage of spores inhibited from germinating	
			Unwashed slides	Washed slides
Untreated	.....	.....	3.5	3.0
Micronized sulphur	6.0	15	78.5	5.5
		60	91.0	26.0
		120	100.0	82.0
Micronized sulphur + S. E. C. Oil[b]	6.0	15	73.5	53.0
		60	100.0	86.0
		120	100.0	100.0
Red copper oxide	2.0	15	25.5	25.5
		60	44.5	45.0
		120	76.5	75.5
Red copper oxide + S. E. C. Oil	2.0	15	26.0	26.0
		60	43.5	40.5
		120	61.0	.....
Jap Beetle Spray	2.5	15	57.5	18.0
		60	100.0	56.0
		120	100.0	70.0
Jap Beetle Spray + S. E. C. Oil	2.5	15	59.5	58.0
		60	100.0	100.0
		120	100.0	100.0
Fermate	2.5	15	76.0	79.5
		60	90.0	88.5
		120	100.0	100.0
Fermate + S. E. C. Oil	2.5	15	82.0	80.5
		60	97.5	94.5
		120	100.0	100.0

[a] The glass slides were coated with nitro-cellulose acetate.
[b] S. E. C. Oil is a self-emulsifying cottonseed oil. It was used at the rate of 1–1600.

Results. Fermate and the Jap Beetle Spray were about equally toxic on unwashed slides, whereas red copper oxide and micronized sulphur were only $\frac{1}{3}$ to $\frac{1}{2}$ as potent (Table 1). On washed slides, the spore counts show that the micronized sulphur spray deposit was reduced in effectiveness to a greater extent than any of the other materials. The Jap Beetle Spray too lost considerable effectiveness when washed. In contrast, Fermate and the red copper oxide showed only small differences in toxicity between the

[6] Heuberger, John W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.

washed and unwashed slides. Loss of toxicity following washing is interpreted as due to partial removal of the original spray deposits.

The addition of S. E. C. Oil significantly increased the retention of the Jap Beetle Spray and of the sulphur, and made for an inhibitory residue equivalent to that of the unwashed slides. In the case of the Fermate and red copper oxide, however, the use of S. E. C. Oil made little difference in their ability to inhibit germination.

Orchard Tests

Methods. All the sprays were applied with a power sprayer to mature orchard trees, of Windsor, Schmidt, Black Republican, Black Tartarian, and Giant varieties. The trees had received the following spray schedule: A dormant application of two per cent tar oil; four applications of flotation sulphur paste at 6–100 (6 pounds in 100 gallons of water), at the green-tip, petal-fall, shuck-split, and shuck-fall stages (the latter two sprays also contained arsenate of lead for curculio); two cover sprays for cherry fruit fly control, the first consisting of micronized sulphur, 4–100, plus rotenone, and the second of micronized sulphur, 2–100, plus rotenone and one-half pint of S. E. C. Oil.

Fermate, Jap Beetle Spray, and Spergon were tested at two concentrations: ½–100 on fruit about ready for harvest and 1–100 on fruit which was to remain on the trees for a longer period of time. The first pre-harvest spray was applied June 13. Micronized sulphur, 2–100, was used as a standard of comparison. S. E. C. Oil, ½ pint to 100 gallons, was used with all the fungicides.

Disease Control on Fruit at Time of Picking

Results. Fruits from Schmidt, Giant, and Black Republican cherry trees, sprayed with the Fermate and the Jap Beetle Spray had not over 5 per cent rot 2 weeks after treatment (Table 2). Most of this decay, especially on the Giant variety, was the result of infections on cracked fruits. Rotted fruit on the sulphur-sprayed trees ranged from 19 per cent on the

TABLE 2.—*Effectiveness of sulphur and organic materials in pre-harvest sprays for controlling brown rot and gray mold on sweet cherries*

Variety	Pre-harvest treatment[a]	Percentage of fruit rotted 2 weeks after treatment[b]
Schmidt	Micronized sulphur 2–100	19
	Fermate 1–100	2
	Jap Beetle Spray 1–100	0
Giant	Micronized sulphur 2–100	38
	Fermate 1–100	5
	Jap Beetle Spray 1–100	2
	Spergon 1–100	33
Black Republican	Fermate 1–100	0
	Jap Beetle Spray 1–100	0

[a] S. E. C. Oil, one-half pint to 100 gallons, was included in all treatments.
[b] A total of 4.07 inches of rain fell between the pre-harvest application and the time the results were taken.

Schmidt variety to 38 per cent on the Giant. Spergon gave no better control than the sulphur. The nontreated cherries in each variety were completely diseased.

Much of the rot that developed on the early varieties was gray mold caused by *Botrytis cinerea*, which developed during the 2-week interval between spray and harvest in which 4 inches of rain furnished favorable conditions for rot development.

Additional data on the value of the Jap Beetle Spray were obtained from an application of this material, ½–100, plus ½ pint of S. E. C. Oil to a block of Windsor trees (a later variety) that had received the pre-harvest spray of micronized sulphur, 2–100, 3 weeks earlier when the other varieties were

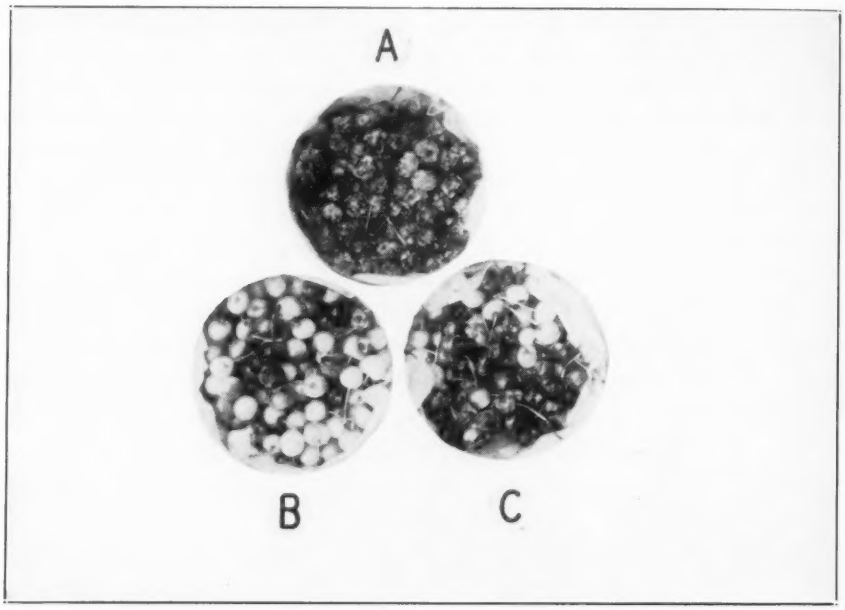

FIG. 1. Brown-rot infection on Napoleon cherries after 8 days in a moist chamber at room temperature following immersion in a spore suspension of *Sclerotinia fructicola:* A. No spray; B. Fermate ½–100; C. Micronized sulphur 2½–100.

sprayed. The fruit was picked a week after treatment during which time almost two inches of rain fell. This additional spray reduced the percentage of rotted fruit from 9.4 to 2.7.

Field tests in 1942 substantiated the data of 1941. One pre-harvest application of Fermate, ½–100, plus ½ pint of S. E. C. Oil, to Napoleon cherries one week before harvest lowered the percentage of rot from 92 per cent, as on nonsprayed trees, to 52 per cent. Two applications of the Fermate, the above and one 17 days earlier, reduced the amount of rot at harvest to 18 per cent; whereas a similar plot receiving two applications of micronized sulphur, 5–100 and 2½–100, respectively, had 41 per cent of the fruit infected with brown rot (Fig. 1). The comparatively high percentage of decay in these plots is no doubt due to the long interval between applications during which 2. 23 inches of rain fell.

The data from two seasons' field trials show that the degree of superiority of Fermate over the standard sulphur spray in the pre-harvest application depends on the number and thoroughness of the previous applications and the environmental conditions.

Disease Control on Fruit After Picking

Although the sweet cherry may leave the orchard apparently sound, serious loss from brown rot and gray mold often develops during the period of transportation and marketing. Since the pre-harvest spray treatment affects their keeping quality, tests were made to compare the effectiveness of the different materials in this regard.

Samples of sound fruits, taken from the various pre-harvest spray plots, were immersed in a spore suspension of *Sclerotinia fructicola* or *Botrytis cinerea*. This inoculated fruit was then placed in moist chambers, consisting of 600-ml. beakers lined with moist paper towels and covered with waxed paper. They were held for incubation periods of 3 to 6 days at room temperature (75°–85° F.).

Protection Against Brown Rot. Fruit from Black Tartarian trees, which had just been sprayed with Fermate, Jap Beetle Spray, and Spergon, ½–100, plus S. E. C. Oil (½ pint–100), was practically all sound after 3 days in the moist chamber, while the unsprayed fruit was 38 per cent rotted (Table 3, Ser. 1). After 6 days, the unsprayed fruits were all infected, while the sprayed fruits showed only from 24 to 32 per cent decay.

The Windsor trees, which had received the late pre-harvest treatment of Jap Beetle Spray, ½–100, plus S. E. C. Oil, had 17 per cent of their fruits decayed after 3 days in the moist chamber, as contrasted with 49 per cent for the check (Table 3, Ser. 2). This count included fruits that had cracked by water-absorption in the moist chambers, which opened an avenue for infection regardless of the spray treatment.

Fermate, 1–100, applied to Schmidt, Giant, and Black Republican trees two weeks before picking, held the percentage of rotted fruit after 6 days in the moist chambers to a low level, when compared with fruits from trees receiving sulphur, 2–100, or no pre-harvest treatment (Table 3, Ser. 3). This heavier dosage of the organic fungicide significantly improved the protection of the inoculated fruit through the post-harvest moist treatment (Table 3, Ser. 1 and Ser. 3). The fruits on this block had been washed by 4 inches of rain before they were picked. Attention is called to these results particularly, because it furnishes an excellent illustration of the ability of the Fermate to resist washing.

Protection Against Gray Mold. Fruits treated with Fermate and Jap Beetle Spray, 1–100, or micronized sulphur, 2–100, developed practically no gray mold after 3 days in the moist chamber, whereas the Spergon-sprayed fruits at 1–100 and the nontreated fruits showed 22 and 36 per cent decayed fruits, respectively (Table 4). S. E. C. Oil (½ pt.–100) was used with all treatments.

At the end of 6 days, Fermate was the most effective material with not

TABLE 3.—*Effectiveness of sulphur and organic materials in pre-harvest sprays in preventing after-harvest brown rot infections on sweet cherries*[a]

Series and variety	Pre-harvest treatment[b]	Percentage of fruit rotted after incubation period of	
		3 days	6 days
Series 1[c]			
Black Tartarian	Nontreated	38	100
"	Fermate ½–100	2	24
"	Jap Beetle Spray ½–100	0	24
"	Spergon ½–100	2	32
Series 2[d]			
Windsor	Nontreated	49	.....
"	Jap Beetle Spray ½–100	17	.....
Series 3[e]			
Windsor	Nontreated	.....	100
Schmidt	Micronized sulphur 2–100	.....	64
Giant	Micronzied sulphur 2–100	.....	54
Schmidt	Fermate 1–100	.....	5
Giant	Fermate 1–100	.....	0
Black Republican	Fermate 1–100	.....	11

[a] Picked fruits were inoculated with spores of *Sclerotinia fructicola* and kept in moist chambers.

[b] One-half pint of S. E. C. Cottonseed Oil was included in all pre-harvest sprays.

[c] Fruit in this series was picked after a light shower the same day it was sprayed.

[d] This treatment was made a week before harvest and the fruit was washed by two inches of rain during that interval.

[e] These treatments were made two weeks before harvest and the fruit was washed by 4.07 inches of rain during that interval.

over 6 per cent of the fruit infected that reflects greater retention during washing. The Jap Beetle Spray and sulphur were about equally effective, allowing 18 per cent of the fruit of the Giant variety to become decayed. The fruits treated with Spergon and the nontreated were 76 and 94 per cent infected, respectively.

TABLE 4.—*Effectivness of sulphur and organic materials in pre-harvest sprays in preventing after-harvest gray mold infections on sweet cherries*[a]

Variety	Pre-harvest treatment[b,c]	Per cent of fruit rotted after incubation period of	
		3 days	6 days
Windsor	Unsprayed	36	94
Schmidt	Micronized sulphur 2–100	4	8
Giant	Micronized sulphur 2–100	2	18
Schmidt	Jap Beetle Spray 1–100	0	2
Giant	Jap Beetle Spray 1–100	4	18
Republican	Jap Bettle Spray 1–100	0	8
Schmidt	Fermate 1–100	0	0
Giant	Fermate 1–100	0	4
Republican	Fermate 1–100	2	6
Giant	Spergon 1–100	22	76

[a] Picked fruits were artificially inoculated with spores of *Botrytis cinerea* and kept in moist chambers.

[b] One-half pint of S. E. C. Oil was included in all pre-harvest sprays.

[c] These treatments were made two weeks before harvest and the fruit was washed by 4.07 inches of rain during that interval.

FRUIT CRACKING

Sweet cherries treated with sprays containing self-emulsifying cottonseed oil were observed to have less cracking than those that were unsprayed (Table 5). The reduction in cracking is attributed to the cottonseed oil.

TABLE 5.—*Effectiveness of Jap Beetle Spray and S. E. C. Oil in reducing brown rot and cracking of cherries*[a]

Variety	Additional pre-harvest treatment	No. of fruits counted	Percentage of fruit cracked	Percentage of fruit rotted
Windsor	Nontreated	300	14.7	9.4
Windsor	Jap Beetle Spray ½–100, plus ½ pint S. E. C. Oil	474	5.5	2.7

[a] This fruit was washed by 1.97 inches of rain between the pre-harvest spray and harvest.

The presence of this oil in the various sprays seemed to form an invisible coating over the fruit, that did not permit absorption of rain water. Oil-sprayed cherries which were immersed in water after picking cracked just

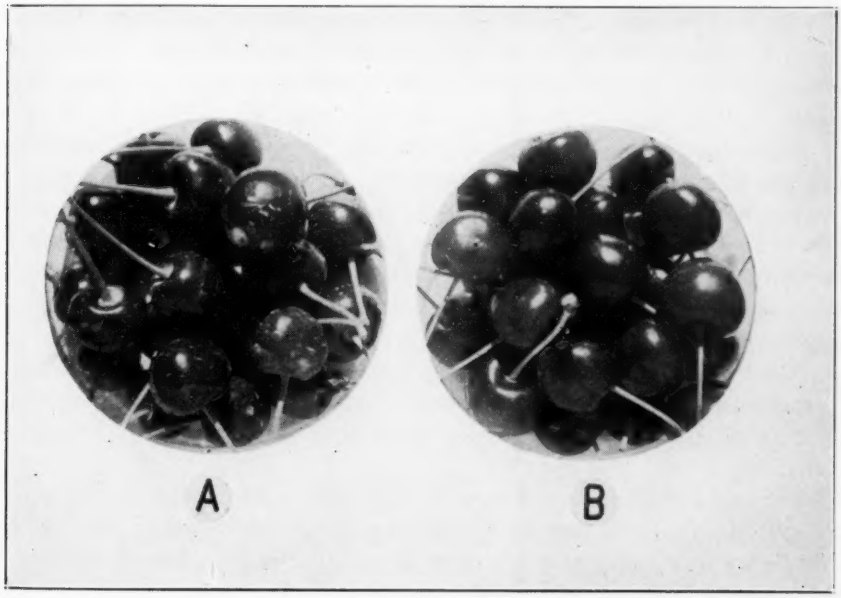

FIG. 2. Comparison of visible residue on Schmidt cherries after spraying with: A. Micronized sulphur 2½–100 plus SS–3 1½ ounces. B. Fermate 1–100 plus S. E. C. Oil ½ pint.

as much as the unsprayed fruits, indicating that the oil functions only to shed the rain.

RESIDUES

Data from the seasons of 1941 and 1942 indicate that Fermate, 1–100, and Jap Beetle Spray, 1–100, are the most effective and desirable of the pre-harvest fungicides tested.

Fermate used alone leaves a black, spotty coverage on cherry fruits, but when a suitable spreader such as S. E. C. Oil, ½ pint, is used with it, the residue is not objectionable on any of the dark varieties of cherries (Fig. 2). On white varieties, Fermate can be used with satisfactory results up to the final pre-harvest application at which time Jap Beetle Spray, 1–100, or wettable sulphur, 2½–100, with a suitable spreader-sticker, may be substituted to avoid the dark residue on the light fruit. Under certain conditions, as where growers with mixed varieties wish to make frequent applications to protect each variety as it reaches maturity, it may be possible and desirable to reduce the concentration of Fermate to ½–100 with the expectation of good results.

SUMMARY

The organic materials, Fermate and Jap Beetle Spray, when sprayed on cellulose-nitrate-coated glass slides were found to be equally toxic against the spores of *Sclerotinia fructicola*. These two materials exhibited two to three times greater toxicity than micronized sulphur or red copper oxide, which have been used for controlling brown rot on sweet cherries. Washing of the sprayed slides before applying the spores indicates the greater retention of the Fermate and red copper oxide over that of Jap Beetle Spray or sulphur. The addition of soluble cottonseed oil markedly increased the amount of toxic residue retained by Jap Beetle Spray and sulphur-sprayed slides.

Fermate and Jap Beetle Spray were superior to Spergon and micronized sulphur when tested as pre-harvest sprays on sweet cherries for control of brown rot and gray mold. The data indicate that the Fermate and Jap Beetle Spray should be used at 1–100, a concentration which could be expected to give appreciable leaf-spot control. These materials at ½–100 have been used with success when more than one application is made or when applied during the harvest period.

Moist-chamber studies and observations made in the market show that Fermate used as a pre-harvest spray enhanced the keeping quality of the cherries on the fresh fruit market more than any of the other materials tested.

Fermate to which a good spreader has been added avoids the objectionable visible residue caused by sulphur sprays on dark colored cherries, but this dark spray material shows more than Jap Beetle Spray or sulphur on light colored fruit.

An emulsified vegetable oil has been found a desirable addition to the pre-harvest spray both from the standpoint of rendering the residue less visible and reducing the amount of cracking.

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A TENDENCY TO ESCAPE TOBACCO-MOSAIC DISEASE IN DERIVATIVES FROM A HYBRID TOMATO

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The Chilean tomato, *Lycopersicon chilense* Dun.,[1] became available as a potential source of resistance to diseases of tomato in 1937, through discovery of a viable seed in an approximately 12-year-old herbarium specimen (3). Studies were begun at once to determine its susceptibility to tobacco mosaic, a disease to which all tested varieties and strains of the garden tomato, *L. esculentum* Mill., have proved susceptible and with which all have been readily infected by abrasive methods of inoculation. The results of these studies, briefly reported already in an abstract (4), have shown that the Chilean tomato is characterized by a tendency to escape infection and that this klendusity is of a heritable nature, being found also in certain derivatives of the hybrid *L. esculentum* × *L. chilense*. The purpose of this paper is to present in detail the evidence on which these conclusions are based.

KLENDUSITY OF THE CHILEAN TOMATO AND SOME OF ITS HYBRID PROGENY

The Chilean tomato proved difficult to infect with tobacco-mosaic virus (*Marmor tabaci* H.). When once infected, however, it developed a systemic chlorotic-mottling type of disease.

The F_1 hybrid *Lycopersicon esculentum* ♀ × *L. chilense* ♂ (3), although intermediate between the parental species in general appearance and especially in fineness of leaf-cutting, was characterized by vigor of vegetative growth. The F_1 plants proved intermediate in susceptibility, escaping infection somewhat more often than ordinary tomato but much less often than *L. chilense*. When infected, they showed chlorotic mottling much like that developing in infected plants of the two parent species. Plants of this first hybrid generation were of low fertility. Their pollen, however, proved adequate to produce a backcross generation.

Plants of the backcross generation, *Lycopersicon esculentum* ♀ × (*L. esculentum* × *L. chilense*) ♂, were exceedingly variable in appearance, showing almost every conceivable combination of the foliage and fruit characteristics of the two original parental species. They were not inoculated with tobacco-mosaic virus, since it appeared probable from the manner of their origin that they would share the relatively high susceptibilities of the F_1 hybrid and of *L. esculentum*. Instead, they were permitted to set open-pollinated fruits in the greenhouse insofar as they were capable of doing so.

[1] In a recent revision of the genus *Lycopersicon* by Muller (U.S.D.A., Misc. Publ. 382) the Chilean tomato is treated as the variety *dentatum* (Dun.) Muller of *L. peruvianum* (L.) Mill. Pending confirmation of the indicated relationship, however, the writer prefers to consider it as a separate species. Typical representatives of the species *L. peruvianum* have not shown comparable facility of hybridization with *L. esculentum* thus far.

Some proved highly fertile, others less fertile, and a few appeared sterile. Seeds obtained from a number of the individuals were planted.

Some sets of seedlings secured in this way as segregating offspring from individual plants of the backcross (B_1) generation appeared on the whole much like the cultivated tomato; others showed characters obviously resembling those of *Lycopersicon chilense*. All were tested by inoculation with tobacco-mosaic virus. Unfamiliarity with the degree of klendusity to be anticipated in the segregating populations may have resulted in failure to detect what later would have been readily recognizable tendencies to escape disease in individuals in some of these populations. However that may be, only one individual capable of escaping disease in the manner of the Chilean tomato was identified. It greatly resembled the wild species in leaf shape but was more vigorous in growth and larger in size. It was the only seedling obtained from one particular plant of the first backcross generation.

This klendusic seedling did not fruit for some time. Finally, after about two years in the greenhouse, it produced and matured a considerable number of fruits as a result of open pollination. The fruits were a little larger than the $\frac{3}{8}$- to $\frac{1}{2}$-inch fruits of the Chilean parent, were marked with a much less conspicuous purple stripe, and displayed a yellow, rather than a cream, color when fully ripe. From 0 to 4 seeds were produced in each fruit; from the first 153 fruits exactly 153 well formed seeds were obtained and planted. From them 12 seedlings grew. Of these, 6 became infected in early tests; whether they were klendusic or not was not properly determined, adequate technique for determining klendusity not being available at the time. Of the remaining seedlings, 3 grew well enough to be available for more extensive tests. When their leaves were rubbed in the presence of juice expressed from mosaic tobacco leaves, after dilution such that ordinary tomato plants would generally but not always become infected in comparable tests, all 3 seedlings showed the tendency to escape infection that had been noted both in the Chilean tomato from which they had originated and in their immediate parent, the lone seedling from a backcross plant.

Identification of the tendency to escape infection in the parental generation and in two successive generations following hybridization and backcrossing appeared to be adequate proof of the heritability of this desirable characteristic. Its mode of inheritance could not be investigated at the time because of low fertility among available derivatives from the hybrid *Lycopersicon esculentum* × *L. chilense*.

EXPERIMENTS WITH SEEDLING 1196C2

The effectiveness of the tendency to escape infection was tested under a variety of conditions in 1 of the 3 seedlings above mentioned. This plant, which will be referred to by its record number, 1196C2, was propagated for more than a year by rooting successive lots of tip cuttings. It differed strikingly from *Lycopersicon chilense* in its more vigorous growth and in

having foliage less finely divided than that of most varieties of the garden tomato, *L. esculentum*.

Plants of 1196C2 and of Bonny Best tomato, in all cases grown from cuttings, were inoculated by rubbing the tips of 3 leaves of each with successive dilutions of juice freshly expressed from a mosaic plant of Turkish tobacco, *Nicotiana tabacum* L. Figure 1 shows the results of this and a subsequent experiment. In the figure, individuals infected in this experiment are represented by the use of black symbols, circles for plants of 1196C2, and triangles for Bonny Best control plants. It will be observed that all plants of 1196C2 were infected in the test with undiluted juice, clearly showing the essential susceptibility of this clone of plants, but that few were infected by the 1 : 10 and none by the 1 : 100 or higher dilutions. On the other hand, the Bonny Best tomato plants were all infected in tests with undiluted juice and the 1 : 10 dilution, many by the 1 : 100 dilution, and some by the 1 : 1000 dilution. Had more leaf surface been rubbed on each plant, somewhat less concentrated inocula might have proved effective both for the ordinary tomato and for 1196C2 plants.

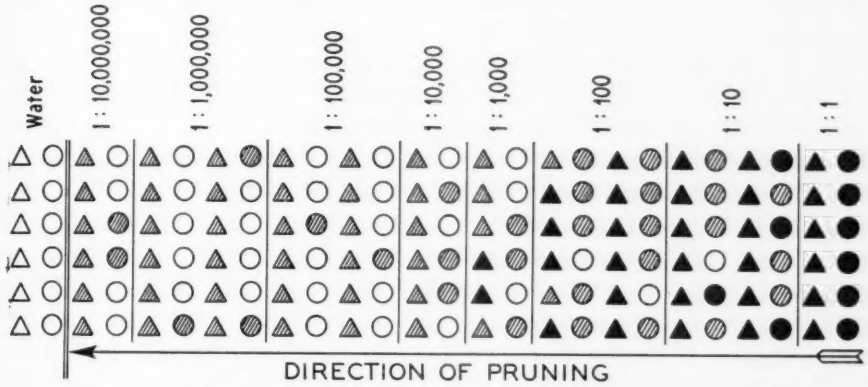

FIG. 1. Results of rubbing and subsequent pruning inoculations of Bonny Best plants (triangles) and 1196C2 plants (circles), as described in text. Black symbols indicate plants that became infected by the initial rubbing inoculation with the diluted juice samples specified. Shaded symbols indicate plants infected only by the subsequent pruning, which proceeded from right to left along each row (last two plants in each row not pruned). Unshaded symbols indicate plants that were not infected by either of these operations. Alternation of plant types on a greenhouse bench is indicated.

It was concluded from this experiment that samples of juice from mosaic plants of tobacco would be most serviceable as inocula for distinguishing klendusic from fully susceptible plants if diluted somewhat more than 1 : 10 but less than 1 : 100 with water. An intermediate dilution, 1 : 50, was tested and proved particularly suitable. It often infected none of the 1196C2 plants in a particular experiment but all of the Bonny Best plants used as controls. This dilution was used thereafter to identify klendusic plants.

A tendency to remain healthy after contaminative superficial contacts, such as those furnished by rubbing inoculation, would not necessarily imply resistance in the presence of other kinds of virus transfer. Cutting back of

vines and pruning of branches on successive diseased and healthy tomato plants constitute a type of inoculation commonly occurring as a result of field and greenhouse practices. These procedures involve wounding of tissues well below the surface of the plant. It was thought that if the klendusity of plant 1196C2 should be dependent on some peculiarity of surface cells alone, cutting operations might render the protective mechanism ineffective. To test this point, the plants earlier used for testing different concentrations of inoculum were all cut back to within 2 inches of the ground, beginning in each row at the end where heaviest inoculations had been applied and most infections had occurred and proceeding toward the end where all plants had escaped infection. Plants originally inoculated with water alone were not included and remained healthy throughout the course of the experiment. The plants to be pruned were cut successively with a razor blade that was cleaned by washing with soap and water only after the completion of each row. The blade was not cleaned during the cutting of successive plants within each row except as the cutting operation itself tended to wipe away virus obtained from diseased plants. The cutting instrument became thoroughly contaminated in removing the tops of the first few plants in each row and, in consequence of this, infected all of the Bonny Best tomato plants that it subsequently cut; nevertheless, more than half of the interspersed plants of 1196C2 escaped. This will be seen from the record presented in figure 1, in which plants infected by this pruning operation are indicated by shaded symbols. All of 42 newly exposed Bonny Best plants and 30 of 62 1196C2 plants were infected. If only exposed plants not immediately preceded by diseased individuals are considered, all of 42 Bonny Best tomato plants and only 8 of 36 1196C2 plants became infected. It may be concluded from this and the preceding experiment that the klendusity of 1196C2 plants was effective in the presence of both rubbing and cutting types of inoculation.

Tendency to escape infection from deep wounding operations suggested that the mechanism responsible for klendusity in 1196C2 plants was present in many and perhaps all cells of the plant and was not confined to superficial layers. The nature of this mechanism was not obvious, however. Some light was thrown on the problem by an observation that infected plants of 1196C2, although they became conspicuously mottled, served poorly as sources of inoculum for infection of *Nicotiana glutinosa* L. Transfers from 6 mosaic Bonny Best tomato plants to 6 plants of *N. glutinosa* resulted in an average of 1175 lesions per test plant; comparable transfers from 6 mosaic plants of 1196C2 yielded only 352 lesions per plant. In each case the source plants had been infected for 7 weeks and were showing distinct chlorotic mottling when used.

DEMONSTRATION OF INHIBITOR

The coupling of conspicuous mottling with what appeared to be low yields of virus in infected plants of 1196C2 suggested that juices of the diseased

plants might be inhibitory rather than actually low in virus content. The question arose, therefore, whether the juices from healthy 1196C2 plants might tend to restrain formation of lesions on inoculated plants of *Nicotiana glutinosa* in the presence of virus from another source. That this was actually the case was shown by inoculating with 3 mixtures, each containing 1 part of juice expressed from mosaic Bonny Best tomato plants diluted with 9 parts of one of the following: water, healthy 1196C2 juice, and healthy Bonny Best tomato-plant juice. Each sample was tested by inoculating 3 plants of *N. glutinosa*. The mixture with water induced the formation of 264, 152, and 420 lesions, that with juice of 1196C2 plants 38, 40, and 38 lesions, and that with Bonny Best juice 285, 243, and 205 lesions. The striking reduction in numbers of lesions caused by using juice from healthy plants of 1196C2 demonstrated that some decidedly inhibitory agent was present in this juice. It suggested that the nature of the juice might have played an important part in preventing some 1196C2 plants from becoming infected in earlier experiments and might have accounted for the apparently low virus content of the individual plants that were infected. If juices of 1196C2 plants act in as inhibitory a way upon infection of their own tissues as upon infection in tests with *N. glutinosa*, it is unnecessary, perhaps, to seek further for a plausible explanation of klendusity in these plants, since it may be assumed safely that some juices are always liberated from an inoculated surface at the moment of inoculation. These juices may be sufficient to affect virus or infected cells, or both, in such a way as to prevent subsequent development of disease. It is possible, of course, that some klendusic plants later may be found not to possess an inhibitory juice. Should this be the case, another mechanism would have to be sought to explain the observed klendusity. Tentatively, however, the phenomenon may be considered as probably attributable to some inhibitory constituent or constituents of expressed juice.

It was thought that advantage from the inhibitory effect of 1196C2 juice might be greater under some circumstances than the inoculating and pruning experiments thus far described had indicated. Contaminative contacts under field conditions of tomato culture are likely to involve virus from within rather than from outside the planting. To test what might be expected to happen if klendusic plants could be substituted for ordinary types of tomatoes in field practice, a greenhouse experiment was devised, in which mosaic plants of 1196C2 and a succession of healthy individuals of the same clone were handled in a way simulating the handling contacts of plants taken from a seed bed for transplanting. For controls, diseased and healthy Bonny Best tomato plants were manipulated in a similar manner. Plants of each kind were arranged in sets of 6 healthy individuals, each set accompanied by a mosaic plant of its own type. For the first test, the right hand was used to grasp successively the infected 1196C2 plant and its 6 healthy counterparts, and simultaneously the left hand was used in the same way for Bonney Best controls. Then both hands were washed thoroughly with

soap and water and the process reversed to compensate for any differences in manner of making contacts with right and left hands. In the second test, the left hand was used for 1196C2 plants, the right hand for Bonny Best controls. The same thorough washing of hands and reversal of procedure was practiced before making the third and fourth tests. Results of this experiment on contaminative handling contacts were striking. None of 24 exposed 1196C2 plants, but all of 24 exposed Bonny Best plants, became infected. The klendusic tendencies possessed by this derivative from the hybrid *Lycopersicon esculentum* × *L. chilense* appeared likely to be highly effective under conditions resembling those in field plantings.

It cannot be predicted yet whether the characteristic of klendusity inherent in plant 1196C2 and its immediate relatives can be transferred to ordinary varieties of tomato, nor whether such transfer if accomplished would be compatible with high quality and yield of fruit. During the period of experimentation, plant 1196C2 has blossomed less abundantly than its immediate parent, which was also klendusic. It has not yet set fruit, though now more than a year old. Naturally it is hoped that sterility will not be complete, for this would require a return to plants of an earlier generation for the production of the additional and more fertile stocks needed to permit investigation of the mode of inheritance of klendusity.

DISCUSSION

Klendusity with respect to inoculation through superficial abrasions and the types of injury involved in pruning operations obviously would not assure that there would be a tendency also to escape infection by insect feeding, should efficient vectors of tobacco-mosaic disease be encountered under field conditions. From time to time suspicion has rested on hypothetical insect vectors as possibly responsible for spread of tobacco-mosaic disease in tomatoes on certain farms where other detectable means of spread have seemed inadequate to account for the observed incidence. It seems probable, however, that any insect transmission that may exist will be difficult to demonstrate under field conditions until spread by mechanical means can be minimized or eliminated. Continued experimentation with klendusic derivatives from the *Lycopersicon esculentum* × *L. chilense* hybrid would seem to be justified even in the absence of knowledge of how such plants may be affected by insect transmission. Production of horticulturally useful klendusic varieties, if feasible, would constitute a step toward elucidation of the still nebulous insect-vector problem in connection with tobacco-mosaic disease in tomatoes in the field, all past experimental work on the subject with other than negative results having been performed under laboratory conditions (1, 2).

The tendency to escape disease encountered in *Lycopersicon chilense* and its derivatives stands in contrast with a trait that has been described as protective in *L. hirsutum* Humb. and Bonpl. This second protective characteristic, discovered by Porte, Doolittle, and Wellman (5), provides a

passive reaction to infection. It is characterized by no hindrance to infection, by high activity of juice from diseased plants, and by absence of obvious signs of disease. *L. chilense* and its klendusic derivatives present a very different picture, involving a tendency to escape infection, a low activity of juice from diseased plants, and clear symptoms of disease in any plants that become infected. *L. hirsutum* derivatives, if responding like the parent species, would escape damage themselves but would constitute a concealed reservoir of virus if infected. *L. chilense* derivatives would tend to remain healthy, *i.e.*, virus-free, but if infected would respond much as ordinary tomatoes do. Both mechanisms may prove valuable for reducing the often underestimated effects of tobacco-mosaic disease in commercial plantings of tomatoes.

SUMMARY

A heritable characteristic of klendusity with respect to abrasive and pruning types of inoculation with tobacco-mosaic virus has been found in the Chilean tomato, *Lycopersicon chilense* Dun., and in certain derivatives from its hybrid with the cultivated tomato, *L. esculentum* Mill. Whether this tendency to escape disease can be transferred to and incorporated in ordinary tomato varieties, and that without interfering with quality or yield of fruits, remains to be demonstrated.

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A TECHNIQUE TO COMPARE VIRULENCE OF ISOLATES OF ALTERNARIA SOLANI ON TOMATO LEAFLETS

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The early blight (*Alternaria solani* (E. & M.) J. & G.) of the tomato (*Lycopersicon esculentum* Mill.) has long caused serious losses. It is recognized that, in general, Alternaria leaf blight has become in recent years probably the most important tomato defoliation disease. Spraying or dusting with Bordeaux mixture or other copper fungicides is the common control measure for the trouble in the field, but the practice is expensive and the application of Bordeaux mixture to tomatoes at times may have an injurious effect on the plants (3, 4, 11). The desirability of horticulturally acceptable tomato varieties that are resistant to Alternaria is, of course, readily appreciated and need not here be discussed. Furthermore, spraying uses strategic materials, both in spray chemicals and in the spraying or dusting equipment, that are required in the present war emergency. All this has given further impetus to increased effort of experiment station workers toward the development of Alternaria-resistant varieties of tomatoes that may replace the present susceptible commercial varieties. The technique herein described was developed as part of a program leading to such an end.

Rands (7), Kunkel (5), Bonde (2), as well as others, have observed that *Alternaria solani* is quite variable in cultural characters. Bonde (2) seems to have been the only worker who has made an extensive study of the differences that may be found in the virulence of isolates of the organism, and his work dealt only with tests on potato.

The technique described by the writer is dependent upon the standardization and control of as many factors as possible. The most favorable conditions for the growth of Alternaria and for the leaf-spot attack are known (6, 7, 8), and these were corroborated before being incorporated into the experimental procedure.

MATERIALS

A single commercial strain of Marglobe tomato was used as the standard host for these studies. It was found that the reactions to inoculum on leaflets of this strain were very consistent. Other strains of Marglobe, however, as well as other varieties, such as Bonny Best, Globe, and Pritchard, were also about equally susceptible to attack.

A number of tests of isolates of *Alternaria solani* were made to compare the severity of disease produced by 7 of the most widely differing isolates that had been collected from typical, naturally occurring Alternaria spots on tomato plants from Maryland fields. The specific identity of these cultures was further established by examinations of both spore and cultural characters by Charles Drechsler and me, and by proof of pathogenicity.

Two culture media were used. First, an agar medium I devised according to the following formula: agar 12 g., water-ground white corn (maize) meal 12 g., cane sugar 12 g., tap water 1000 cc., autoclaved at 15 pounds' pressure for 20 minutes, and used without straining. Second, the Tochinai liquid medium (9, 1).

The incubation chambers in which the growing inoculated plants were held were of the type commonly used in such phytopathological studies. They were mounted on a bench in a warm greenhouse, had slanting glass tops shaded with cheesecloth, and a layer of wet sphagnum moss in the bottom. The air temperature inside the chambers ranged from 23 to 27° C.

PREPARATION OF CULTURES FOR INOCULUM

The best method of growing Alternaria isolates for inoculation purposes were studied. After tests with both liquid and agar media it was found that the most satisfactory results were from inoculum grown on agar. A piece of young fungus mat about 15 mm. in diameter was planted in the middle of an agar plate. After the colony had grown to about 50 mm. in diameter, 6-mm. disks were cut from just inside its edges as standard bits for inoculum.

It was found practically impossible at times to handle some cultures in certain series so that they would be available to compare with other types of the organism that grew more slowly in cultures. Experiments showed, however, that when colonies had grown to the best size for use as inoculum, the Petri-dish cultures could be refrigerated (6 to 8° C.) in a moist chamber and kept for at least two weeks, after which they could be used for inoculum without any appreciable change in relative virulence of the organism.

METHODS OF INOCULATION AND INCUBATION OF PLANTS FOR VIRULENCE TESTS

Determination of the comparative virulence of isolates of an organism is manifestly dependent on the results of tests on inoculated host plants. Many different methods were used in making these tests. Leaflets were inoculated on growing plants, and excised leaves and leaflets also were inoculated. Both attached and excised leaflets were then incubated in moist chambers, in light and in darkness, and under a variety of other controlled environments. While in certain instances tests with excised parts were promising, there also were unexplained failures. Often soft-rot bacteria invaded excised leaflets and inhibited and disturbed Alternaria-infection results. It also was believed that pathogenicity or virulence determinations on an excised host organ might perhaps be less conclusive than kindred observations on the same organs attached to growing plants.

The plants most successfully employed in these studies were grown in a warm greenhouse and were ready for use when they had reached the 5-leaf stage, which was usually about a month after sowing. Seedlings were transplanted into 3-inch pots at the time true leaves appeared as a small bud between the well developed cotyledons. The soil used was moderately fertile greenhouse compost without additional enrichment.

Plants were selected for inoculation that had two full-grown primary or "smooth" leaves at the base above the cotyledons. The first adult or "rough" leaf above the primaries was considered as No. 3. Its nearly expanded terminal leaflets were chosen for inoculation. In preliminary work it was found that results on primary leaflets were irregular, and that lesions on leaf No. 4 tended to be smaller than those on leaf No. 3.

In all tests, plants were selected for uniformity from a large population and arranged in rows in the inoculation chamber. They were oriented so that the tip of leaf No. 3 was pointing in one direction for ease of access by the operator, and rows were so spaced that no inoculated leaflets touched each other or the top or sides of the chamber. The plants were thoroughly watered before inoculating, particular attention being given to gentle washing of the leaflets that were to be used for test purposes. These leaflets were atomized both before and after inoculation.

The technique employed by Andrus *et al.* (1) in their work on Alternaria leafspot resistance in tomato was investigated. This method satisfactorily demonstrated relatively wide differences in virulence between isolates; but, when finer distinctions were sought, the results were too irregular and likely to be confused. Observations at the Bureau of Plant Industry Station, Beltsville, Maryland, indicated that unavoidable mechanical injuries from top-dipping and the variations in quantity and placement of inoculum were the main sources of these irregular results.

Plants grown in pots were watered and arranged in the incubation chamber as described above. A small spatula made from a piece of stiff platinum wire (B. & S. gauge 16) was used to transfer disks of inoculum from Petri-dish cultures onto the plants. If the leaflets were dry, plants were atomized with water lightly just before inoculation and a disk was placed fungus side up on the upper side and at the center of each leaflet. Plants were invariably atomized again lightly at the time the chambers were closed. Inoculated leaflets were examined each day for disease progress.

In judging the right time to record results, a standard culture of known virulence was always used for reference purposes. Data were taken when a majority of leaflets inoculated with this culture had reached the most severe stage of injury; that is, when leaflets were fully involved but remained attached to the main petiole, when necrosis of petiolules had occurred with severe epinasty of the whole conpound leaf, and when about a third of the infected leaves had dropped because of disease effects. The incubation period varied from 4 to 7 days, but the most clear-cut data were generally secured at the end of 5 days.

Tests on plants handled as described are fairly economical of space and time. In one chamber 2 ft. 9 in. wide by 15 ft. long, 200 plants were easily accommodated for inoculation purposes. A very satisfactory test unit consisted of 10 plants on each of which 3 leaflets were inoculated, thus giving 30 numerical estimates of virulence for each test. It is believed that 5 plants per test would be satisfactory in some experiments. It took two

workers about 4 hours to arrange and inoculate 380 plants (1140 leaflets inoculated) in the incubation chambers, and less than two hours to estimate and record relative virulence observed on these plants.

PROGRESS OF SYMPTOMS OF ALTERNARIA LEAF ATTACK

It was imperative to know the changes from day to day in appearance of a typical alternaria-infected leaflet. These changes were recorded as the basis for the numerical evaluation of relative virulence. The progress in symptoms of Alternaria leaf spot was studied under both field and laboratory conditions. Notes of disease progress on inoculated plants, were made 3 times daily in the greenhouse, while observations in the field were made once a day. In the greenhouse, rapidity of attack differed and it would seem was correlated with the relative virulence of the isolates used for inoculum. However, the symptoms always followed a consistent course.

TABLE 1.—*Typical observed progress of macroscopic symptoms of Alternaria solani attack of an inoculated leaflet on a Marglobe tomato plant, under controlled experimental[a] conditions*

Time observed	Symptom appearance (macroscopic)		Epinasty effects	Virulence value[b]
	Of inoculum	Of invaded area		
Day 0, afternoon	Inoculum applied	No infection	None	0
" 1, morning	" powdery	.....	.....	0
" 1, noon	" pubescent	.....	.....	0
" 1, afternoon	Hyphae penetrating leaf	Faint stippling	Questionable tilt of leaflet	1
" 2, morning	"	Irregular narrow ring	Petiolule deflected	3
" 2, noon	Aerial hyphae collapsed	Wider ring	Slight deflection of whole leaf	3
" 2, afternoon	"	Zone broader	Whole leaf pushed downward	4
" 3, morning	"	Infected spot 18 mm. diameter	"	6
" 3, noon	"	Infected spot 20 mm. diameter	"	7
" 3, afternoon	"	Spot same size; necrotic fleck along vein	"	8
" 4, morning	"	Spot same size; necrotic fleck along vein; leaflet tip collapsing	"	10
" 4, noon	Hyphae growing on surface	Spot same size; several necrotic flecks along vein; collapse extending to base	"	12
" 4, afternoon	"	Spot same size; necrotic flecks along vein; whole leaflet necrotic	"	13
" 5, morning	Aerial hyphae very abundant	Leaflet shriveled	Abscission	(14)

[a] See accompanying text of this paper for methods employed in this study and controlled environment involved.

[b] For these virulence values, see diagrams in figure 1 and the description of those diagrams in the text.

Table 1 is a record of successive appearance of the symptoms of one typical spot as observed on one leaflet in a single experiment. This succession of appearances has been found in general to occur in the field, although the spots started as much smaller lesions, were slower in development, and were often arrested at a comparatively early stage on the mature leaves under the less sheltered, drier, outside conditions.

It will be seen in table 1, that first macroscopic symptoms of infection in the greenhouse appeared at about the close of 24 hours of incubation. In an equal period following, there was slow extension of a border of tissue darkened by fungus attack around the inoculum-disk. During the overnight period that followed the first 48 hours of incubation, the most rapid spread of the directly invaded, darkened leaf area was observed. Following this, small, irregular, necrotic areas were soon found along and between the veins in the more remote portions of the leaflet, even back into the petiolule, and sometimes affecting adjacent inoculated leaflets. Microscopic examination and isolation studies disclosed no fungal growth in these spots appearing at some distance away from the main leaf lesion. Increasing general flaccidity of the lamina followed appearance of these latter systemic effects; and this ended in shriveling of the leaves bearing the severely diseased leaflets.

In my work on fusarium wilt of tomato (10) epinasty appeared among the earliest symptoms. Infection of tomato leaflets by Alternaria also causes an epinasty, apparent very soon after the first symptoms of leaf attack are macroscopically visible. Under the described greenhouse conditions, epinastic effects increase in severity along with increase in the foliar lesion and end in dropping (abscission) of the whole compound leaf if the terminal leaflets are very severely affected. Leaflets by themselves are not dropped.

NUMERICAL EVALUATION OF DISEASE SEVERITY ON INOCULATED LEAFLETS

The usual progress of symptoms under a standard set of conditions was determined from many studies. On the basis of this progression, comparisons were made of the amount of disease caused by isolates that differed in various ways, and a numerical grading of virulence was then devised. The grades were readily recognizable, and these grades or values are diagrammatically represented in figure 1 (see also table 1 for comparison of disease progress with numerical values).

An inoculated leaflet showing no infection was given the value 0. The first sign of infection was stippling about the inoculation disk, valued at 1 or 2, depending upon severity. When a discrete, moderately narrow collar of infecting mycelium developed on a narrow band of darkened tissue around the inoculation disk, the infection was valued at 3. Infection from 1 to 3, inclusive, was considered "mild." As the infection spread beyond the delimited collar, the discolored area increased and the different comparative widths were given the "medium" values of 4, 5, 6, and 7. At 7 there

appeared to be an approximate maximum point beyond which little if any further expansion of the dark colored infected area occurred around the inoculum. The more virulent isolates caused symptoms that appeared as small to large isolated water-soaked areas in the leaflet tissue, often some distance from the seat of infection. When these occurred the "medium severe" values 8, 9, and 10 were applied, depending upon intensity of effect. As infection increased to "severe," the water-soaked areas enlarged and the

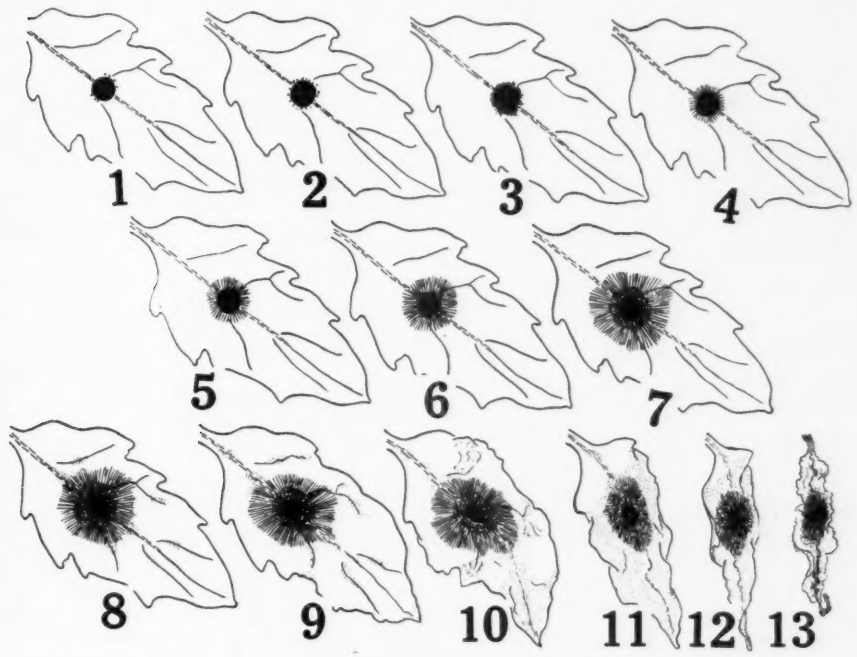

FIG. 1. Diagrammatic representation of progressive stages in macroscopic symptoms of attack on tomato leaflets after inoculation, using a disk cut from a culture of *Alternaria solani*. The disk is represented by black spot at center of leaflet. Virulence values were based on 14 stages of attack as follows: No infection (not illustrated) = 0; few small spots around inoculum = 1; ring of small infection spots around inoculum = 2; spots coalesced into narrow, irregular dark halo = 3; width of halo about one-third diameter of inoculum = 4; halo width slightly less than diameter of inoculum = 5; halo about same as inoculum diameter = 6; halo somewhat wider than diameter of inoculum = 7; few small water-soaked flecks outside halo = 8; somewhat more advanced than 8, small tip portion of leaflet flaccid = 9; coalescence of water-soaked spots, half of leaflet wilting = 10; water-soaking and wilting of about three-fourths of leaflet = 11; all leaflet wilted except small areas at base = 12; leaflet entirely wilted and shriveled = 13; like 13 but whole compound leaf dropped (not illustrated) = 14.

leaflets became flaccid at the tip. Finally, the whole leaflet, except the base near the petiolule was involved, and hung limply. These reactions were valued at 11 and 12. When a limp leaflet was found shriveled and crinkled around the edges it was valued at 13. In the "very severe" infections, the whole compound leaf to which 3 diseased terminal leaflets were attached would drop, and this being an extreme severity, it has been suggested that it might be given a value of 14.

TABLE 2.—*Results of successive tests*[a] *on Marglobe tomato plants inoculated with isolates of Alternaria solani to demonstrate comparative virulence and small difference of reaction between isolates*

Test number and date started	Index of virulence obtained from isolate[b] indicated:						
	A	B	C	D	E	F	G
	Mean	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*
1 May 11	10.1 ± 0.15	9.5 ± 0.18	4.3 ± 0.20	7.4 ± 0.20	.....	1.7 ± 0.22	.....
2 May 25	10.0 ± 0.21	10.2 ± 0.01	4.0 ± 0.32	.....	.....	.....	.....
3 June 8	10.1 ± 0.20	18.8 ± 0.39	3.7 ± 0.18	.....	4.4 ± 0.25	1.4 ± 0.09	.....
4 June 22	12.3 ± 0.19	12.1 ± 0.26	3.9 ± 0.26	.....	.....	.....	.....
5 July 13	9.0 ± 0.01	11.0 ± 0.32	4.7 ± 0.25	.....	.....	.....	1.6 ± 0.02
6 August 10	11.8 ± 0.01	9.8 ± 0.24	2.8 ± 0.02	6.8 ± 0.24	4.9 ± 0.02	1.5 ± 0.10	.....
7 August 24	9.0 ± 0.26	11.1 ± 0.26	3.3 ± 0.02	6.3 ± 0.27	4.6 ± 0.22	1.6 ± 0.11	1.7 ± 0.02
Mean	10.8 ± 0.11	10.6 ± 0.11	3.8 ± 0.09	6.8 ± 0.16	4.6 ± 0.13	1.5 ± 0.10	1.7 ± 0.01

[a] For technique involved see description given above in this paper. Readings were recorded from 30 leaflets for each test, from which the mean (index of virulence) with its standard error were obtained. For diagrams representing approximate virulence values see figure 1. Compare also with table 1.

[b] Isolates selected after preliminary trials of relative disease-producing capacities and found to be different in degrees of virulence.

CONSISTENCY OF RESULTS IN REPEATED TESTS

In developing the technique incident to the study of relative differences in virulence of Alternaria isolates on tomato leaflets, it is essential that a test of this kind be regularly reproducible, and that virulence readings be consistent in relative magnitude on successive trials. Consecutive tests were run (Table 2), using the 7 isolates as sources of inoculum and following the experimental technique already described. These isolates were selected on the basis of their proved wide range of difference in severity of disease effects.

Each isolate was recultured at least twice during the series of tests. A new series of plants and cultures was grown for each test. Three of the isolates were relatively stable, grew well in culture, and were tested 7 times during 4 months. Fewer tests were made with the others because of slower growth or the need for more repeated reculturing due to notably wide variations in cultural characters.

From table 2 it is evident that standard errors were small, results were reasonably consistent from one test to another, and dependable comparisons could be made of virulence indices. The largest differences between isolates are obvious: A and B, having mean indices of about 10.5 contrasted with F and G having mean indices at about 1.5. Smaller differences are also demonstrable: C having a mean index of $3.8 \pm .09$ compared with D having an index of $6.8 \pm .16$; or E having an index of $4.6 \pm .13$ compared with F having an index of $1.5 \pm .10$. Close comparisons should be attempted between single evaluations of isolates only when they run within a single test. Similarly, limited numbers of repeated evaluations should be compared only when involved in completely comparable sets or tests.

SUMMARY

A laboratory technique was devised to test the virulence of isolates from leaf lesions of tomato early blight (*Alternaria solani*).

Tomato seedlings, about a month old, were grown under standard conditions, and leaflets attached to plants were selected for testing virulence of isolates.

Cultures of the pathogen were grown on agar under standard conditions and standardized pieces of the cultures were used for inoculum.

The course of symptoms of attack was studied under controlled conditions of incubation and compared with field observations.

Progressive symptoms on diseased leaflets were classified according to numerical grades, and these numbers were used as virulence values.

Controlled inoculation studies were made and data were compared statistically. Reactions were found to be reasonably consistent from one test to the next, and the relative virulence was expressed on a numerical basis.

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USTILAGO STRIAEFORMIS. I. GERMINATION OF CHLAMYDOSPORES AND CULTURE OF FORMA AGROSTIDIS ON ARTIFICIAL MEDIA[1]

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The widespread occurrence of *Ustilago striaeformis* (Westd.) Niessl. on different species of grasses has led several authors to investigate the life history of the organism. An interesting feature of these investigations is the discrepant reports regarding the germinability of fresh smut chlamydospores and the difficulty of securing cultures of the organism on artificial media. Davis (1, 2, 3, 4), made extensive collections of *U. striaeformis* on *Agrostis alba* L., *Phleum pratense* L., *Poa pratensis* L., *and Poa annua* L. None of the collections of fresh chlamydospores that he tested germinated more than a small percentage. As a result, Davis investigated factors that influenced germination of the smut chlamydospores. From the work so conducted, he concluded that an after-ripening period was prerequisite for successful germination. Despite the fact that he tried many times, Davis failed to secure cultures from after-ripened chlamydospores.

Fischer (5), working with a race of *Ustilago striaeformis* that occurred on *Agropyron pauciflorum* (Schw.) Hitchc.[2] and *Elymus glaucus* Buckl. experienced no difficulty in germinating fresh chlamydospores of the organism. The race with which Fischer worked grew readily on artificial media and produced numerous sporidia. The cultural studies conducted by Fischer represented the first successful cultivation of *U. striaeformis* on artificial media.

During the past year, germination tests were conducted with chlamydospores from smutted plants of *Agrostis alba*. Most collections of diseased plants failed to yield germinable chlamydospores; however, one of the collections provided spores that germinated 50–75 per cent. Since an abundant source of germinable chlamydospores was supplied by this collection, it was used for most of the studies embodied in this report.

MATERIALS AND METHODS

During the fall of 1941, 49 smutted plants of *Agrostis alba* were transplanted to a greenhouse for observation. The diseased plants were removed from 49 sod plugs obtained from 12 widely separated pastures located in the central part of Pennsylvania. After each plant was established in a 4-inch pot, fresh chlamydospores from sori in the leaves were tested for germinability.

[1] Contribution No. 43, of the U. S. Regional Pasture Research Laboratory, Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the northeastern states.

[2] Now known as *A. trachycaulum* (Link) Malte.

All germination tests were conducted in quadruplicate on microscope slides that contained paraffin wells. The wells were prepared by dipping an 18 mm. glass ring into melted paraffin. The glass ring was then touched to a slide and a paraffin ring left superimposed. The process was repeated so that four paraffin wells were deposited on each microscope slide. By placing the wells along one side of the slide, sufficient space was left below each well for labeling.

RESULTS OF GERMINATION TESTS

When fresh chlamydospores from each of the 49 plants were tested for germination, two plants from one pasture yielded chlamydospores that germinated 50–75 per cent. The plants of this collection, designated 14A, were obtained from a pasture near Lamar, Pennsylvania, on October 24, 1941. Tests of 11 smutted plants subsequently secured from different parts of this same pasture revealed that fresh chlamydospores from 7 of the plants germinated 1–75 per cent, while fresh chlamydospores from the remaining 4 plants failed to germinate. None of the diseased plants collected in other pastures provided more than an occasional germinable chlamydospore.

EFFECT OF DISTILLED AND TAP WATER ON GERMINATION OF CHLAMYDOSPORES

Since collection 14A provided an excellent source of germinable chlamydospores, tests were conducted to determine what influence different kinds of water had on germination of spores. This was done to determine, if possible, the reason why most chlamydospores of *U. striaeformis* forma *agrostidis* W. H. Davis fail to germinate in a fresh state.

For each test, 3 kinds of water were used; tap water, water distilled in a Pyrex glass still and water distilled in a tin still. A drop of water was placed within each paraffin well and chlamydospores were scraped from sori on diseased leaves and dispersed in the individual drops. Each slide so prepared was then placed in a Petri dish moist chamber and incubated at room temperature for 16–24 hours.

Excellent germination occurred in each of the three kinds of water. This eliminated the possibility that water used for germination tests contained substances likely to inhibit germination of the smut spores.

When germinable chlamydospores were placed in drops of water and each drop was covered with a coverglass, germination failed to occur, suggesting that the spores required free access to atmospheric conditions despite the fact that many spores germinate below the surface of uncovered drops of water.

INFLUENCE OF ALCOHOL AND ETHER ON GERMINATION OF CHLAMYDOSPORES

Davis (1) discovered that the after-ripening period of smut chlamydospores could be decreased appreciably by treating the spores with chloroform and citric acid. This suggested the possibility that non-germinable

spores of *Ustilago striaeformis* might be treated with different agents and induced to germinate without first subjecting them to an after-ripening period. Preliminary tests were therefore conducted to determine what effect alcohol and ether had on germinable chlamydospores of *U. striaeformis.*

Chlamydospores from collection 14A were treated with 95 per cent ethyl alcohol. Microscopic examination of spores so treated revealed that the spores collapsed almost instantaneously. When the treated spores were placed in water, they regained their turgid condition and appeared uninjured. Germination tests revealed that momentary treatment with 95 per cent ethyl alcohol failed to injure the spores. Longer treatments of 15–30 seconds duration caused complete inhibition of germination despite the fact that the collapsed spores returned to a turgid state.

When germinable chlamydospores of *Ustilago striaeformis* were placed in di-ethyl ether, they survived treatments of several minutes duration despite the fact that the spores collapsed. Germination was reduced considerably by the longer treatments but a short exposure to ether failed to decrease subsequent germination.

Chlamydospores that failed to germinate in a fresh state were then procured from a similar series of *Agrostis alba* plants and treated with ethyl alcohol and ether. All attempts to stimulate germination by treatment with these agents failed.

EFFECT OF AGE AND POSITION OF SMUT SORI IN THE LEAF ON SPORE GERMINATION

Davis (1) concluded that germination of fresh smut spores from different grasses was not dependent on age of the leaf. He found, however, that the after-ripening period was shorter for spores removed from dead leaves than for spores removed from green leaves. Davis also demonstrated that spores from different sori within a leaf varied with respect to germinability and length of after-ripening period. With this in mind, tests were conducted to determine whether or not similar conclusions could be drawn from the material at hand.

Smut spores from leaves of different plants of collection 14A were tested for germinability. The minimum germination encountered was 1 per cent while the maximum was 75 per cent. A wide range in germination was also discovered when chlamydospores from different plants within a clone were tested for germinability. Variation in germination to the same extent was encountered when similar tests were conducted on different smutted leaves from a single plant. In keeping with Davis' contention, age of leaf was found to be of minor importance in germination of the smut chlamydospores.

Spores were next removed from numerous sori along the entire blade of a leaf and tested for germination. The results confirmed those of Davis who found that spores from different sori in the same leaf varied in ability to germinate. Further tests revealed that mature spores removed from ruptured or unruptured sori or even from different parts of the same sorus differed markedly in ability to germinate.

INFLUENCE OF STORAGE TEMPERATURE ON SURVIVAL OF CHLAMYDOSPORES

Leaves from smutted plants of collection 14A were stored dry at room temperature, 10° C., and 5° C. to determine what effect storage temperature had on germinability of chlamydospores. Spores in leaves stored 4 days at room temperature failed to germinate. Smutted leaves stored at 10° C. for 30 days yielded germinable chlamydospores, however, germination was reduced from an original 75 per cent to less than 10 per cent. Chlamydospores from leaves stored at 5° C. survived 28 days with a similar reduction in germinability. These tests demonstrated that fresh, germinable spores of *U. striaeformis* were capable of surviving at least 30 days when stored at favorable temperatures.

CULTIVATION OF USTILAGO STRIAEFORMIS FORMA AGROSTIDIS ON ARTIFICIAL MEDIA

Since excellent germination of fresh chlamydospores occurred in drops of water, the possibility of securing germination on agar media was investigated. For this purpose, drops of 3 per cent potato-dextrose agar were placed aseptically on sterile coverslips. Spores from smutted leaves were next transferred as aseptically as possible to the agar drops, and the coverslips were inverted on glass rings in Petri dish moist chambers.

After 24–36 hours incubation at room temperature, the agar drops inoculated with chlamydospores were examined for spore germination. Each germinated spore usually displayed a single branched or unbranched promycelium 20 to 50 μ long. No sporidia were observed although lateral branches resembling sporidia were frequently noted on rapidly growing promycelia.

Single germinated chlamydospores were then removed from the agar drops by means of a micromanipulator and transferred to fresh drops of agar. About 25 per cent of the chlamydospores so handled continued their growth and developed into strictly mycelial colonies. A typical germinated spore with lateral mycelial branches is shown in figure 1, A. The mycelial type of growth secured in cultures of *U. striaeformis* forma *agrostidis* is a marked contrast to the sporidial type of growth obtained by Fischer (5) from *U. striaeformis* forma *hordei* G. W. Fischer.

Of 10 single-chlamydospore cultures secured from diseased plants of *Agrostis alba*, two different biotypes were observed. Colonies of one form were characterized by a flat, lustrous, ridged growth that was a light buff[3] on potato-dextrose agar. This colony-form is illustrated in figure 1, B. Colonies of the other form were slower growing and were of a fluffy, raised mycelial type of growth that was also light buff. A representative of this colony-form is shown in figure 1, C.

The effect of different temperatures on growth of the cultures was determined by growing the organism in 250-cc. flasks that contained 50-cc. aliquots of potato-dextrose agar. Each culture was grown at 7 different

[3] Ridgway, R. Color standards and color nomenclature. 43 pp., 53 colored plates. (Washington, D. C.). 1912.

temperatures arranged at 5° intervals from 5° C. to 35° C. Best growth occurred at 20° C. and 25° C. No growth occurred at 35° C. and only feeble growth at 5° C.

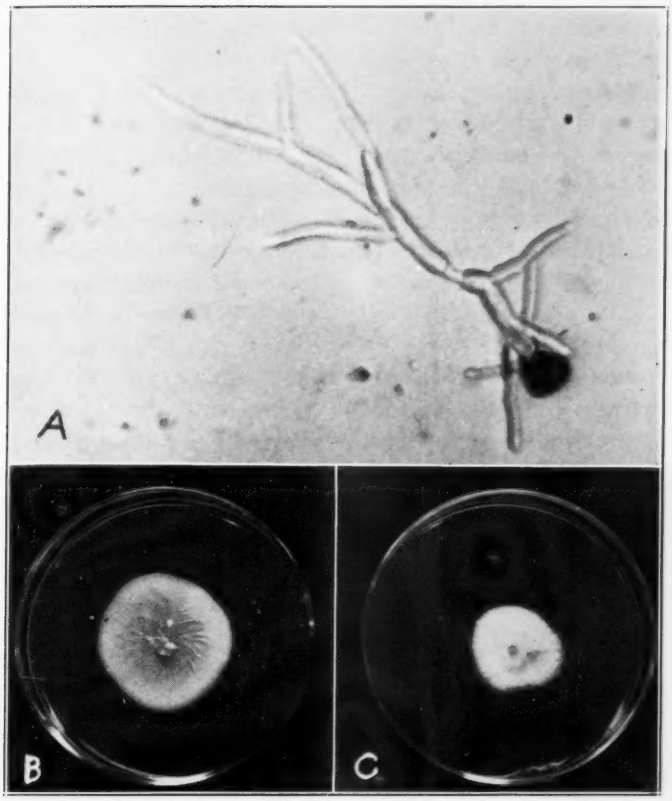

FIG. 1. Germinating chlamydospore and cultures of *Ustilago striaeformis* forma *agrostidis*. A. Chlamydospore after 72 hours on potato-dextrose agar. ×636. B and C. Cultures on potato-dextrose agar in 90-mm. Petri dishes. Growth after 19 days at 27° C.

DISCUSSION

The discovery that an occasional collection of *Ustilago striaeformis* forma *agrostidis* is capable of germinating immediately when removed from the plant gives added incentive to the investigation of why most collections of this smut fail to germinate unless they are subjected to an after-ripening period. It seems probable that the collection that germinated readily, in contrast to the majority of collections that fail to germinate, is a physiologic form or strain within the race *U. striaeformis* forma *agrostidis*, although the data available at present do not eliminate the possibility of a differential effect of the host plant upon this character. The germinable strain may be further subdivided into biotypes based on cultural characteristics as indicated in this paper.

The question then arises whether or not *Ustilago striaeformis* is in reality a collection of species and should be reclassified with respect to natural hosts and inoculation studies on related and unrelated hosts. Work by Davis, although not conclusive showed that chlamydospores of *U. striaeformis* taken from timothy failed to infect redtop when cross-inoculation tests were carried out with these and other grasses. On the basis of inoculation tests, spore size, and spore morphology, Davis concluded that distinct biological races of the smut occurred on *Agrostis alba*, *Phleum pratense*, *Poa pratensis* and *Poa annua*. Fischer's extensive inoculation tests (5), demonstrated that the race of smut with which he worked readily infected species of *Agropyron*, *Elymus*, and *Hordeum* but failed to infect species of *Poa*, *Phleum*, *Agrostis*, etc. Only the weight of further experimental evidence will demonstrate whether or not *U. striaeformis* is an assemblage of species or forms.

SUMMARY

A collection of *Ustilago striaeformis* forma *agrostidis*, the chlamydospores of which germinate without an after-ripening period, is described.

Chlamydospores selected from the same sorus or from different sori on the same plant vary in ability to germinate.

Single-chlamydospore cultures of this race of the organism were grown on artificial media.

Growth of the cultures was strictly mycelial in form and could be differentiated into 2 biotypes.

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DISAPPEARANCE OF VIRUS FROM MOSAIC-DISEASED SUGARCANE PLANTS

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(Accepted for publication October 26, 1942)

Sugarcane is propagated vegetatively by planting stalks. In varieties very susceptible to mosaic, all plants developing from a diseased seed piece usually show mosaic. In some varieties, however, one or more buds on a diseased seed piece may develop into plants free from mosaic symptoms. This observation has been reported by several workers. Lyon (3), in Hawaii, reported that shoots of the Lahaina cane threw off mosaic during the growing season. Also, healthy plants were observed to develop from stubble pieces of cane that had mosaic the previous season. Kunkel (2) also observed that certain mosaic-diseased stools of sugarcane often recovered. When seed cane was taken from these stools and planted, the young shoots remained healthy for a time, though later some developed mosaic. Kunkel considered this as probably a new infection. East (1), in Cuba, observed much the same thing. He found that some of the plants remained healthy for over a year, while others became infected within 3 months and then recovered again. He set forth two hypotheses that might explain this. The host might kill the virus and throw off the symptoms, remaining partially resistant for a time, or it might reduce the virulence of the virus for a time, in which case symptoms might again appear if the resistance of the host was lowered. Tims and Edgerton (4) in Louisiana reported that the relative resistance of P.O.J. 213 and P.O.J. 228 was to a considerable extent due to the ability of the plants to throw off the disease and to produce virus-free buds. Infected stalks of these varieties, when planted produced both healthy and diseased shoots. The resistant varieties produced a much greater number of healthy shoots than did the susceptible varieties. They reported also that mosaic symptoms commonly disappeared on the resistant P.O.J. canes. Many plants showing the disease in early summer were apparently healthy in the fall. In a later investigation, Tims, Mills, and Edgerton (5) found recovery from mosaic to be common in C.P. 29/320 and C.P. 28/11, and occasional in C.P. 28/19. The infective virus was not always absent from plants which had apparently recently recovered from the disease, since in some cases the disease was produced by inoculating juice from such plants into healthy plants. The percentage of infection, however, was not high.

In Louisiana it has frequently been observed that when mosaic-diseased stalks of sugarcane are planted some buds on such seed pieces develop into plants free from mosaic symptoms. It also has been determined with certain varieties, that mosaic symptoms disappear very commonly in the field from some plants during the growing season. The question naturally arises,—

are such plants free from the mosaic virus, and are they immune from infection by the same virus?

In order to obtain more definite information in regard to the virus condition of sugarcane plants in which the mosaic symptoms had disappeared, it seemed desirable to carry out further tests. The results of experiments to determine whether or not plants without mosaic symptoms, grown from diseased stalks, or plants from which symptoms disappeared during the growing season still contained the mosaic virus, and whether or not such plants were immune from reinfection with the same virus are reported in this paper. The results were largely obtained from inoculation tests.

In all the infection tests the mosaic was transmitted by juice inoculations. Diseased shoots were macerated in a food chopper and the juice extracted from the material by pressure in cheesecloth. Several cc. of juice was placed in the whorl of leaves at the top of the plant and inoculation was effected by pricking through the spindle of the plant with fine needles that carried the virus into the young, growing tissues of the leaves. Under good growing conditions, mosaic symptoms usually showed in the newly developed leaf tissues in two to three weeks. For some unknown reason the percentage of inoculated plants that developed mosaic was often rather low, especially with varieties in which the rate of pick-up of mosaic in the field was slow. As the work was carried on in fields well isolated from mosaic-diseased cane, very seldom were mosaic symptoms observed in plants not inoculated. The period of time during which infection could be obtained by artificial inoculation was limited to the months of May and June. It was practically impossible to obtain satisfactory infection after early July. Whether or not this was due to high temperatures during this portion of the growing season, to high light intensities which might possibly inactivate the virus *in vitro* during the time required for inoculation, or to some other factor is not known at the present time. Due to this fact, however, it was impossible to determine by inoculation whether or not plants from which mosaic symptoms disappeared during the current season were susceptible to reinfection by the mosaic virus.

SUSCEPTIBILITY OF RECOVERED PLANTS

In order to determine whether or not mosaic-free plants grown from diseased stalks were immune from infection, the following experiments were made.

In a test started March 16, 1942, in the greenhouse, 10 symptomless plants of C.P. 28/19, grown from diseased stalks, were inoculated with a virus from C.P. 28/19. As checks, 10 healthy plants of the same variety and 10 healthy plants of the variety Co. 281, grown from healthy stalks, were inoculated with the same virus. Final results were read May 6. Of the 10 symptomless plants inoculated, 6 developed mosaic and 4 no mosaic. Of the 10 check plants of C.P. 28/19 inoculated, all developed mosaic. Of the 10 check plants of Co. 281 inoculated, 8 developed mosaic and 2 no mosaic.

On May 11, 1942, an experiment was made in the field in which 7 symp-

tomless plants of C.P. 28/19 grown from mosaic-diseased stalks were inoculated with C.P. 28/19 virus; and 5 symptomless plants of C.P. 33/243, grown from diseased stalks, were inoculated with C.P. 33/243 virus. As checks, 5 plants of C.P. 28/19 and 5 plants of C.P. 33/243, grown from healthy stalks, were inoculated with mosaic virus from C.P. 28/19 and C.P. 33/243. Final results were read June 25. The 7 symptomless plants of C.P. 28/19 developed mosaic. Of the 5 symptomless plants of C.P. 33/243, 4 developed mosaic and 1 no mosaic. In the checks, 4 of the 5 C.P. 28/19 plants and all 5 of the C.P. 33/243 plants developed mosaic.

From the results obtained it is apparent that sugarcane plants that have recovered from the mosaic disease or symptomless plants that have developed from mosaic-infected seed cane will take the disease when inoculated and are in no sense immune.

ABSENCE OF VIRUS IN RECOVERED PLANTS

In order to determine whether active infective virus was present in sugarcane stalks which had apparently recovered from the mosaic or in stalks that had developed from mosaic-infected seed pieces, a number of tests were carried on during the seasons of 1940, 1941, and 1942. In the tests, juice was expressed from symptomless plants that had developed from infected seed pieces and from plants from which the mosaic symptoms had disappeared. The results of the tests are included in the table. In all experiments, for controls healthy plants were inoculated with virus from mosaic-infected plants.

In the 17 tests (listed under 11 experiment numbers in table 1) in which 425 plants were inoculated with juice from a total of 59 symptomless plants, only one developed the mosaic disease. This one can be assumed to have developed from natural infection. Of the checks inoculated with virus from mosaic-infected plants, 43.1 per cent developed mosaic. The evidence seems to be conclusive that the symptomless plants did not contain active, infective virus.

SUMMARY

Experiments were carried out during 1940, 1941, and 1942 involving inoculations of healthy cane plants with juices from (1) mosaic plants, (2) symptomless plants grown from diseased seed pieces, and (3) plants from which mosaic symptoms had disappeared during the current season.

These experiments comprised seventeen sets of comparisons, involved 6 varieties of sugarcane, and included 790 inoculations.

In each of the 17 experiments inoculations with juices from mosaic canes were followed by a development of mosaic symptoms. The percentage of infection ranged from 10 to 80 per cent.

In 16 of the 17 experiments inoculations with juices from symptomless canes grown from diseased seed pieces, and inoculations with juice from plants from which symptoms had disappeared during the current season

TABLE 1.—*Results of inoculations with (1) juice from symptomless plants grown from mosaic-diseased seed cane, (2) with juice from plants from which symptoms had disappeared during the current season, and (3) with virus from mosaic plants (checks)*

Source of inoculum			Experiment number and date initiated	Inoculation results			
Variety	Number symptomless plants	Number mosaic plants		Varieties inoculated	Number plants inoculated	Number plants developing mosaic	Number plants no mosaic
C.P. 29/320	3		I—May 1940	C.P. 28/70	15	1[a]	14
C.P. 29/320		3		C.P. 28/70	15	2	13
C.P. 28/19	3		I—May 1940	C.P. 28/19	15	0	15
C.P. 28/19		3		C.P. 28/19	15	6	9
C.P. 28/19	3[b]		I—May 1940	Co. 281	15	0	15
C.P. 28/19		3[b]		Co. 281	15	5	10
C.P. 33/243	3		I—May 1940	C.P. 33/243	15	0	15
C.P. 33/243		3		C.P. 33/243	15	8	7
C.P. 33/243	3[b]		I—May 1940	C.P. 28/70	15	0	15
C.P. 33/243		3[b]		C.P. 28/70	15	8	7
C.P. 29/320	4		II—June 1940	C.P. 28/70	20	0	20
C.P. 29/320		3		C.P. 28/70	15	2	13
C.P. 33/243	4		II—June 1940	Co. 281	20	0	20
C.P. 33/243		3		Co. 281	15	3	12
C.P. 29/320	5		III—May 1941	Co. 281	25	0	25
C.P. 29/320		5		Co. 281	25	20	5
C.P. 29/320	5		IV—June 1941	Co. 281	25	0	25
C.P. 29/320		5		Co. 281	25	17	8
C.P. 29/320	5[b]		IV—June 1941	C.P. 28/70	25	0	25
C.P. 29/320		5[b]		C.P. 28/70	25	10	15

TABLE 1.—(*Continued*)

Source of inoculum			Experiment number and date initiated	Inoculation results			
Variety	Number symptom-less plants	Number mosaic plants		Varieties inoculated	Number plants inoculated	Number plants developing mosaic	Number plants no mosaic
C.P. 29/320	5		V—June 1941	Co. 281	25	0	25
C.P. 29/320		5		Co. 281	25	17	8
C.P. 29/320	5[b]		V—June 1941	C.P. 28/70	25	0	25
C.P. 29/320		5[b]		C.P. 28/70	25	10	15
C.P. 33/243	5[c]		VI—May 1941	C.P. 33/243	25	0	25
C.P. 33/243		5		C.P. 33/243	25	17	8
C.P. 33/243	3		VII—June 1941	C.P. 33/243	15	0	15
C.P. 33/243	3			C.P. 28/19	15	0	15
C.P. 33/243		6		C.P. 33/243	30	22	8
C.P. 28/19	1		VIII—May 1941	C.P. 28/19	10	0	10
C.P. 28/19		1		C.P. 28/19	10	4	6
C.P. 28/19	5		IX—June 1941	C.P. 28/19	50	0	50
C.P. 28/19		5		C.P. 28/19	50	5	45
C.P. 29/320	4		X—June 1941	Co. 281	40	0	40
C.P. 29/320		4		Co. 281	40	15	25
C.P. 29/320	6[c]		XI—June 1942	C.P. 28/70	11	0	30
C.P. 29/320		5		C.P. 28/70	11	6	19

[a] Interpreted as natural infection.
[b] Same virus as was used to inoculate other variety in same experiment.
[c] Plants from which symptoms disappeared during the current season.

were not followed by a development of mosaic symptoms. In one experiment symptoms developed on one out of 15 plants inoculated. The development of symptoms under these circumstances was attributed the secondary infection in the field.

The results obtained appeared to establish the fact that no infective virus was present in symptomless plants grown from sugarcane seed stalks originally exhibiting mosaic symptoms, nor in plants from which symptoms disappeared during the current season.

Experiments made in 1942 showed that mosaic-free plants grown from diseased seed pieces were not immune from reinfection by the mosaic virus.

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MOSAIC SPOTS OF FIG FRUITS[1]

IRA J. CONDIT[2] AND W. T. HORNE[3]

(Accepted for publication November 30, 1942)

In earlier papers on fig mosaic,[4,5] brief mention was made of the occurrence of mosaic spots on fig fruits. The wide prevalence of these spots, both on caprifigs and on edible figs, warrants a more detailed account of the manifestations of this disorder.

MOSAIC SPOT OF CAPRIFIGS

In a bulletin[6] entitled "Caprifigs and caprification," published some 20 years ago, there appeared an illustration of an unusual spotting of caprifigs, with the following caption: "This peculiar spot or blemish is a characteristic of some of the profichi . . . of the Samson (Markarian No. 1). It is found on all trees of the variety and is evidently distributed by cuttings." These blemishes marred the appearance of the figs and prevented them from developing properly. Examination of specimens failed to show any pathogen present. Recent observations have confirmed the opinion previously expressed,[4,5] that this spotting of caprifigs is a manifestation of fig mosaic.

Occurrence

The mosaic spot of caprifigs has been observed only on the profichi, which, in the hot interior sections of California, mature early in June. The spot has not been seen on any of the mammoni or summer figs, which follow the profichi, or on the mamme figs, in which the fig insects or blastophagas pass the winter. Nor has any similar spot or blemish been noted on the leaves of these trees, although many of the leaves show mosaic spots and malformations.

The Samson Caprifig. The spot was first found on the Samson caprifig, a variety probably introduced from Asia Minor in 1882, by G. P. Rixford, and established on the Stanford Ranch at Vina, California. From this introduction many cuttings were propagated and distributed by various persons, including Herbert Samson of Corning and Henry Markarian of Fresno. The latter gave it the variety name Markarian No. 1. The mosaic spot has been present on a certain percentage of profichi of every Samson tree examined in the fig districts of California. No similar spot has been

[1] Paper No. 485, University of California Citrus Experiment Station, Riverside, California.

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[4] Condit, Ira J., and W. T. Horne. A mosaic of the fig in California. Phytopath. **23**: 887–896. 1933.

[5] Condit, Ira J., and W. T. Horne. Further notes on fig mosaic. Phytopath. **31**: 561–563. 1941.

[6] Condit, Ira J. Caprifigs and caprification. Calif. Agr. Exp. Stat. Bull. **319**: 341–377. 1922.

found on profichi of Roeding No. 3, Stanford, or any of the dozen other varieties of caprifigs commonly grown.

Profichi of the best varieties of caprifigs set and mature practically 100 per cent of the crop. Samson figs that are affected by spotting invariably drop prematurely; some drop when small, others become malformed and then drop, while a few reach almost full size and color, even though the spotting is present. Noninfected figs are dark green in color until almost fully ripe, and are symmetrical in shape without prominent external blemishes.

A census of the profichi borne by a Samson tree at Riverside showed that 37.2 per cent of these fruits were visibly spotted.

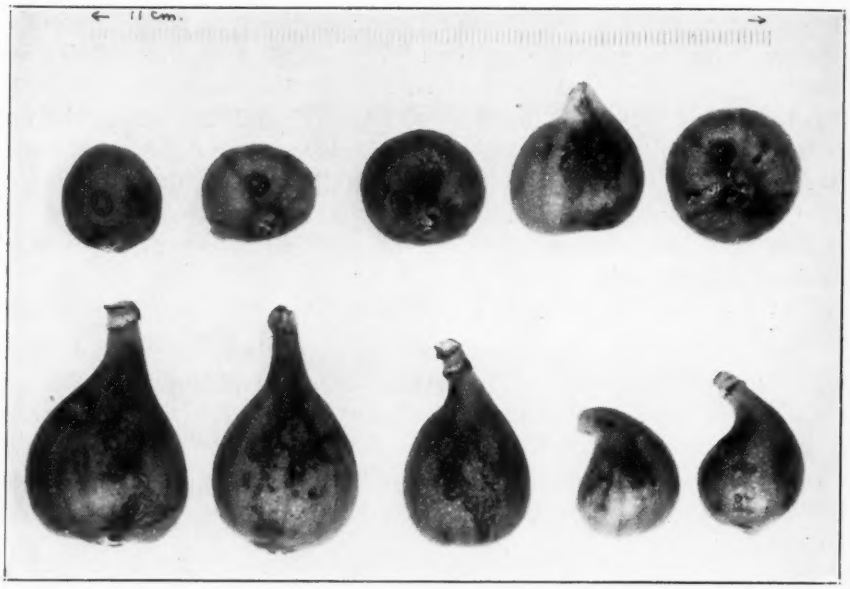

FIG. 1. Mosaic spot of the caprifig has been found only on the Samson variety and on a few seedlings; it is apparently caused by a virus.

Caprifig Seedlings. A few years ago, mosaic spots very much like those on the Samson were found on a seedling caprifig. Since then similar spots have been found on the profichi of 58 caprifig seedlings out of a total population of 4,034 seedlings that have been fruiting at Riverside since 1937. Most of these seedlings were the progeny of 6 common figs pollinated by Roeding No. 1 caprifig, which never shows mosaic spots. No seedling caprifigs of the cross Calimyrna × Samson showed this mosaic spotting of profichi, although it is the Samson on which the spot is so prevalent.

The Mosaic Spot and its Effect on the Fruit

The mosaic spots on caprifigs are first manifest as watery or discolored areas on the body or neck of the fruit. These areas gradually become circular, or crescent-shape, in outline, often forming rings from 1 to 6 mm.

in diameter (Fig. 1). The most distinctive spots are circular, craterlike protrusions, depressed at the center, the whole becoming brownish in color and finally necrotic. Some spots are in the form of concentric circles, the outer circle occasionally as much as 10 mm. in diameter. Other spots are irregular in outline, brownish in color, and bordered by areas deficient in chlorophyll.

The necks of profichi appear to be very susceptible to this spotting. Frequently, the tissues on only one side of the neck are affected, and the result is a characteristically lopsided or crooknecked fruit (Fig. 1).

Laboratory Studies

Tissues of profichi affected by spotting were prepared by the paraffin method, sectioned, and studied under the microscope.

Cells of the parenchyma are the first ones to be affected. In this tissue there appear dense aggregations of cells, often with collapsed walls, as contrasted with the spherical and normal thin-walled cells. The affected cell areas gradually enlarge until they extend from the fibrovascular bundles to the surface of the fruit, and the whole area becomes more or less necrotic.

Spotted fruits of the profichi crop of the Samson caprifig, when cultured, failed to produce any organisms that might be responsible for the spotting. No fungus hyphae were found in thin sections prepared for microscopic examination.

MOSAIC SPOTS OF EDIBLE FIGS

No spots exactly like those found on caprifigs have been noted on edible figs, although at least 3 seedlings have shown fruit with spots somewhat comparable. These 3 seedlings are: No. 32–49, of unknown parentage; and Nos. 61–51 and 61–56, both of the progeny White Genoa × Roeding No. 1. The spots on No. 32–49 are often in concentric rings, although they appear mostly as minute pustules. As shown in figure 2, E, spots on seedling No. 61–51 are unusually prominent and appear as brown rings or as dark circular areas. The spots persist on the mature fruit and somewhat mar the normal coloration.

Mosaic spots on fruits of named varieties show a definite relation to leaf mosaic on the same tree. Thus Mission, which is often badly mosaicked in foliage, shows prominent spotting of the immature fruit (Fig. 2, A). Turkey also shows mosaicked areas, both on leaves and on fruit. On figs of these two varieties, the spots are mostly apparent as sharply defined, light-green circular areas contrasting with the normal dark-green color of the fruit. In some varieties, such as Grise St. Jean, the circular spots may become rusty or necrotic (Fig. 2, B). As previously recorded,[7] mosaic spots on fruits of Brunswick (Fig. 2, C), Celeste, and Sultane, not only cause deformation of the fruit but also bring about premature abscission of the fruit stalk.

[7] See footnote 4.

TRANSMISSION OF FIG MOSAIC

Mosaic spots on fig fruits are assumed to be caused by the same virus that causes mosaic of fig leaves. A preliminary experiment on the transmission of the disease was accordingly attempted.

Plants of tomato and of wild tobacco (*Nicotiana glauca* Graham) were inoculated with the sap from conspicuously mosaicked shoot tips and leaves of Mission fig trees. Carborundum powder was used in these inoculations, substantially as suggested by Rawlins,[8] except that the infusion was diluted

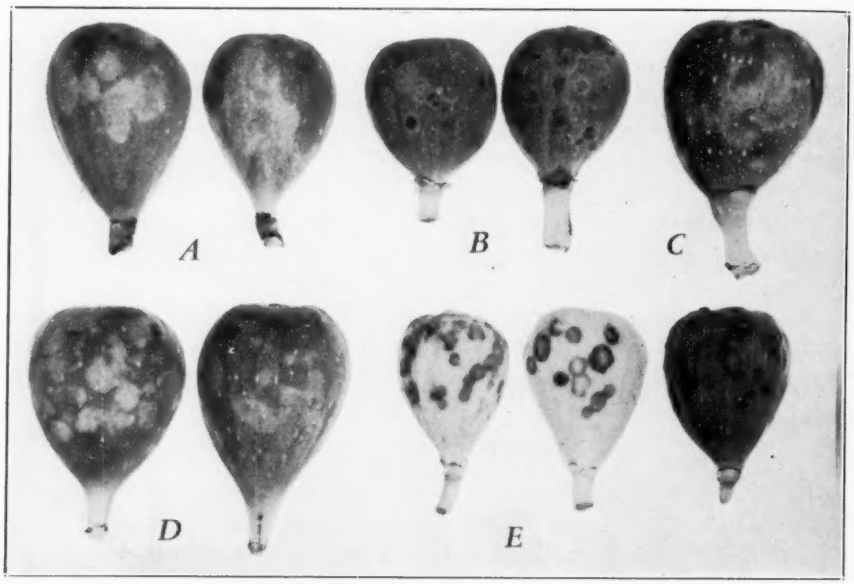

FIG. 2. Mosaic spots on edible figs. A. Mission. B. Grise St. Jean. C. Brunswick. D. Turkey. E. Seedling 61–51.

with distilled water (the amount of water being less than that of crushed tissue) and the leaves of the plants were rubbed rather vigorously with a cotton plug before inoculation. The inoculum was washed off after a few minutes by sprinkling the plants freely. Results were apparently negative. J. M. Wallace kindly assisted in examination of the treated plants.

SUMMARY AND CONCLUSIONS

Over twenty years ago Samson caprifigs of the profichi crop were found bearing blemishes or spots in the form of circular brownish protrusions. These spots were found only on the Samson and appeared on profichi of all trees of this variety examined in California. Affected figs become malformed and drop prematurely. During the past few years, similar spots have been found on 58 caprifig seedlings out of a total population of 4,034

[8] Rawlins, T. E., and C. M. Tomkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.

seedlings fruiting at Riverside. Mosaic spots of caprifigs, when cultured, failed to produce any pathogen that might be responsible for the spotting. This spotting of caprifigs is apparently the result of a mosaic disease rather narrowly restricted as to its host and somewhat peculiar in its expression.

Mosaic spots on edible figs differ somewhat from those on caprifigs. Some are in the form of concentric rings or dark circular spots, while others are apparent as sharply defined, light-green circular areas.

Attempts to transmit leaf mosaic of fig to tomato and wild tobacco were unsuccessful.

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GROWTH SUBSTANCES AND THE RUST FUNGI[1]

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Relationships between the fungi and growth substances have been investigated since the turn of the 20th century, but it is only as chemically pure growth substances were made available that the information has been definite and detailed. Since 1930, research on vitamin requirements of fungi has increased greatly, and a recent review of the literature by Robbins and Kavanaugh[2] lists about 350 fungus species whose vitamin relationships have been studied. The genera *Puccinia* and *Erysiphe* are not mentioned in the report of Robbins and Kavanaugh; and it is only recently that Pryor[3] reported that under certain conditions thiamin increased the development of powdery mildew on cantaloupe. Except for this work on *Erysiphe,* no information is available concerning the vitamin requirements of these obligate parasites. Naturally, one cause has been the impossibility of growing these fungi except on their natural hosts. Another may be the assumption that obligate parasites receive all the necessary vitamins or growth substances from their hosts. Not all hosts are equally suitable for growth of rusts, however. A certain rust may grow well on one host and poorly or not at all on a closely related one. For example, race 17 of *Puccinia graminis tritici* grows well on Marquis but is unable to grow to any extent on Reliance, both *vulgare* wheats. The opposite is true of race 38, which grows well on Reliance and poorly on Marquis. Although it has not been proved that wheat plants contain substances that promote growth and development of fungi, the inability of a rust race to grow within the noncongenial host may be due to absence or to insufficient amounts of proper growth substances. One variety of wheat might contain sufficient quantities of the growth substances required by many of the physiologic races of rust and would be susceptible to those races, while another variety might lack the particular growth-promoting substances needed by a certain race and as a result would be resistant to that race. It seemed possible that supplementing whatever a natural host afforded a rust parasite might enable the rust to make better progress in tissues of a host on which it grew poorly or not at all. Therefore, a number of experiments were made with 4 physiologic races of *Puccinia graminis avenae* on 3 varieties of oats and 7 races of *P. graminis tritici* on several varieties of common and durum wheat, an emmer, and einkorn.

[1] Paper No. 2054 in the Scientific Journal Series, Minn. Agr. Exp. Station.

[2] Robbins, William J., and Virgene Kavanaugh. Vitamin deficiencies of the filamentous fungi. Bot. Rev. 8: 412–471. 1942.

[3] Pryor, Dean E. The influence of vitamin B_1 on the development of cantaloupe powdery mildew. Phytopath. 32: 885–895. 1942.

EFFECTS OF GROWTH SUBSTANCES ON REACTIONS OF SEEDLINGS TO RUST

Only water-soluble growth substances were used in experiments with wheat or oats seedlings and their respective stem rusts. Thiamin chloride, riboflavin, nicotinic acid, ascorbic acid, and beta-indole acetic acid were used alone, in 10^{-4} or 10^{-6} molar concentration, and a mixture of the 5 substances also was used and designated "mixture." Cereal seeds were soaked in the reagents for 24 hours preceding planting. In some experiments each 4-inch pot of seedlings received 75 cc. of the appropriate solution each day, so that the vitamins might be absorbed through the roots. It was expected that such absorbed materials would soon reach mesophyll cells of the seedling leaves and become available to the rust hyphae through their haustoria within the cells. In other experiments vitamins were supplied directly to the mycelium by filling the interstices of the leaf tissue with the solution. A hypodermic syringe fitted with a short, thick-walled rubber tube in place of a needle, as Newton, Lehman, and Clark[4] suggested, was placed against the leaf surface and the vitamin solution was gently forced through the stomata until the interstices were filled and about 2 inches of the tissue on both sides of the rubber tube appeared water-soaked. With slow and gentle pressure, no injury resulted. The water-soaked appearance lasted only a short time, usually less than an hour; but during that time the solution and rust mycelium were in direct contact. In still other experiments rusting seedling leaves were supplied with vitamin solutions by a method used by Gassner and Hassebrauk.[5] Pots of rusting seedlings were inverted over solutions of growth substances, and the leaves remained submerged for several hours at a time to permit a certain amount of absorption through the leaf. Table 1 summarizes the different seedling experiments. Throughout the series there were no consistent changes in development of rust or in seedling host reaction to rust that could be attributed to the treatments with growth substances. There were minor differences in the number of hours required for flecking, in the degree of chlorosis of infected tissues, and even in the abundance of sporulation. The reactions of Khapli emmer to race 15 of *Puccinia graminis tritici* ranged from flecks to type 1 and those of Marquis to its virulent races ranged from type 3 to type 4. Nevertheless, the variations were not consistent in duplicate pots of seedlings nor in repeated experiments. Most of the variations from the checks were within the range of variability normally attributed to slight differences in environment. No host that was normally resistant to the rust race involved became susceptible to that race after vitamin treatment; and there was no definite suppression of rust in hosts that were normally susceptible to the rust race used. The

[4] Newton, R., J. V. Lehman, and A. E. Clark. Studies on the nature of rust resistance in wheat. Can. Jour. Res. (C) **1**: 5–35. 1929.

[5] Gassner, G., and K. Hassebrauk. Über die Beeinflüssung der Rostanfälligkeit durch Eintauchen geimpftes Blätter in Lösungen von Mineralsalzen und anderen Stoffen. Phytopath. Zeitschr. **5**: 323–342. 1933.

water-soluble growth substances apparently exerted no influence on stem rust in wheat and oats seedlings.

Detrimental effects of growth substances. Generally there was very little effect on host or on parasite when the growth substances were supplied to cereal seedlings in different ways. In certain cases, however, nicotinic acid injured wheat seedlings. When a 10^{-4} molar solution was used to fill the intercellular spaces by means of the rubber-fitted syringe, tip burning was

TABLE 1.—*Varieties of wheat and oats inoculated as seedlings with different physiologic races of stem rust and supplied in various ways with growth substance*

Physiologic race of rust		Host variety	Means of supplying growth substances	Reaction[a] to rust
Puccinia graminis avenae	5	Oats Bond	Solution forced into leaf interstices	S
	5	Minrus	do	R
	8	Bond	Solution absorbed through roots	S
	8	Seven-o-three	do	S
	10	Bond	do	S
	10	Seven-o-three	do	R
	11	Bond	do	S
	11	Seven-o-three	do	R
P. gr. tritici	15	Khapli emmer	Solution forced into leaf interstices	R
		Wheats		
P. gr. tritici	15	Marquis	Solution forced into leaf interstices	S
	17	do	Solution absorbed through roots	S
	17	Reliance	do	R
	38	Marquis	do	R
	38	Reliance	do	S
	56	Arnautka	Solution forced into interstices	R
	56	Mindum	Leaves immersed in solution	R
	56	Kubanka	do	R
	56	Einkorn	Leaves immersed in solution	R

[a] Reaction to rust in all these experiments was the same as the reaction expected with each host-parasite combination under normal conditions. S denotes susceptibility; R denotes resistance.

severe on both Arnautka and Marquis wheats. Slight burning occurred when the same concentration of nicotinic acid was furnished for root absorption by wheat seedlings of several varieties. Injury to the host was never noted with any of the other growth substances.

RUST DEVELOPMENT ON OLDER PLANTS

Forcing a urediospore suspension into unexpanded portions of a cereal culm provides an ideal incubator for spore germination and infection of young and succulent host tissues. The severity of infection resulting is high, except when the leaf sheaths are too tightly rolled to permit introduction and distribution of the spore suspension. Urediospores of certain rust races were suspended in vitamin "mixture" solution and then introduced by hypodermic syringe and needle into various parts of culms of field-grown wheat varieties (Table 2). Similar inoculations with urediospores suspended in distilled water served as checks. By providing growth substances

for absorption by the fungus germ tubes, it might be possible to augment the initial vigor of the infecting rust, so that the rust could more easily and quickly parasitize the noncongenial host. Actually, however, no differences in rust development or host reaction were due to the suspension medium. Older parts (leaf sheaths, peduncles, glumes, and awns) of wheat varieties that were susceptible as seedlings to the races of rust with which they were inoculated remained susceptible. Conversely, those that were resistant or immune as seedlings remained resistant or immune under the two types of

TABLE 2.—*Field-grown varieties of wheat inoculated in boot or in a preboot stage with urediospores of different physiologic races of stem rust suspended in solutions of growth substances*

Physiologic race of rust	Host variety	Stage of host inoculated	Reaction[a] to rust
Puccinia graminis tritici 15	Marquis	Preboot	V.S.
		Boot	S.
15	Thatcher	Preboot	M.S.
		Boot	M.S.
15	Hope × Thatcher hybrid	Preboot	V.S.
		Boot	M.S.
17	Ceres	Preboot	M.S.
		Boot	S.
17	Thatcher	Preboot	I.
		Boot	I.
19	Marquis	Preboot	M.S.
		Boot	M.S.
19	Coronation	Preboot	M.S.
		Boot	M.S.
34	Hard Federation	Preboot	V.S.
		Boot	V.S.
34	Hope	Preboot	V.R. to S.
38	Marquis	Preboot	M.R.
		Boot	M.S.
36	Ceres	Preboot	V.S.
		Boot	V.S.
38	Coronation	Preboot	M.S.
		Boot	M.S.

[a] V.S. = very susceptible.
S. = susceptible.
M.S. = moderately susceptible.
M.R. = moderately resistant.
V.R. = very resistant.
I. = immune.

inoculation. Thus, in the field and on older plants, as well as on seedlings, the growth substances used had no effect on stem rust development.

GERMINATION OF SPORES IN GROWTH SUBSTANCE SOLUTION

To determine the effect of growth substances on the germination of rust spores, agar blocks were impregnated with the vitamin solution. Loegering[6] first used relatively large surfaces of 2 per cent water agar to study germinating rust spores, and Mitchell[7] cut blocks of agar and allowed different chemical reagents to diffuse through them so that he could study the effects on germination of rust spores. From hardened 2 per cent agar, blocks ¾

[6] Loegering, William Q. A satisfactory medium for germination of urediospores of *Puccinia graminis tritici*. Phytopath. 31: 952–953. 1941.

[7] Unpublished results of experiments of J. E. Mitchell, Minnesota Agricultural Experiment Station.

inch square and $\frac{1}{8}$ inch thick were cut, then placed in vitamin solution for 24 hours so that the reagents might diffuse through the agar. Impregnated blocks, with excess solution removed by filter paper, were put on glass slides, and urediospores were spread over them before they were placed in a moist chamber. Freshly collected viable stem rust urediospores and also urediospores stored at low temperatures for several weeks or months were used, and spore germination was observed at regular intervals. Within spore lots there were no significant differences in the germination of spores spread over the surface of a plain agar block or spread over the surface of an agar block through which the vitamin "mixture" had diffused. Percentages of spores germinating, time required for germination, and length and vigor of germ tubes were approximately the same in all replicates of a single spore lot.

CONCLUSIONS

From the general negative results of the foregoing experiments it is concluded that the water-soluble growth substances, thiamin chloride, riboflavin, nicotinic acid, ascorbic acid, and beta-indole acetic acid, do not play an important role in the problem of resistance in cereal plants to the obligately parasitic stem rust; and that pathogenic differences in physiologic strains within a rust species may not be attributed to different growth substance requirements.

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A NONCHROMOGENIC SPORULATING VARIANT OF ALTERNARIA SOLANI[1]

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(Accepted for publication January 28, 1943)

Alternaria solani (E. and M.) J. and G., as most frequently isolated in Indiana from tomatoes afflicted with early blight, produces a yellowish to deep-red pigmentation in potato-dextrose agar and sporulates sparingly. A culture, designated S–16, which does not color the agar and produces conidia profusely, arose as a variant from a culture obtained from a single-spore culture obtained from an early-blight lesion on an infected tomato leaf collected at Santa Maria, California, by M. W. Gardner in 1937.

The culture has been very useful in laboratory, greenhouse, and field studies of early blight wherein the use of large numbers of conidia was necessary. It has been found difficult to produce conveniently an adequate number of conidia on the typical *Alternaria solani* cultures by application of methods[2, 3, 4, 5] reported to stimulate sporulation. The S–16 culture also has been useful as a marker in studies designed to determine the role of inoculum introduced on southern-grown tomato seedlings in initiating or increasing the severity of early-blight infection in Indiana tomato fields as distinguished from that inoculum native in such fields.

Such studies were made by atomizing locally grown tomato seedlings, at time of pulling, with a dilute suspension of conidia from the S–16 culture, packing the plants as is done commercially, and storing them for 48 hours before setting. The seedlings became infected in a manner similar to southern-grown seedlings shipped north after becoming contaminated with early-blight conidia at the time of pulling and packing. Near the close of the tomato season, isolations were made from early-blight leaf spots, stem cankers, and infected fruits to determine what proportions were traceable to the S–16 and to native field inocula, respectively.

Bonde[6] has shown that strains of *Alternaria solani* exist on the potato, and that many strains may develop through saltation in culture. During the winter of 1937–1938 several hundred Alternaria conidia were collected on tomato débris in southern, central, and northern Indiana and from aerial spore traps located at Vincennes and at Lafayette, Indiana. Representative

[1] Cooperative investigation of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture and the Department of Botany, Purdue Agricultural Experiment Station. Journal Paper No. 75, Purdue University Agricultural Experiment Station.

[2] Dillon Weston, W. A. R. The sporulation of *Helminthosporium avenae* and *Alternaria solani* in artificial culture. Trans. Brit. Mycol. Soc. **20**: 112–115. 1936.

[3] Kunkel, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. and M. Mem. Brooklyn Bot. Gard. **1**: 306–312. 1918.

[4] Ramsey, G. B., and Alice A. Bailey. Effect of ultra-violet radiation upon sporulation in Macrosporium and Fusarium. Phytopath. (Abstract) **20**: 141. 1930.

[5] Rands, R D. The production of spores by *Alternaria solani* in pure culture. Phytopath. **7**: 316–317. 1917.

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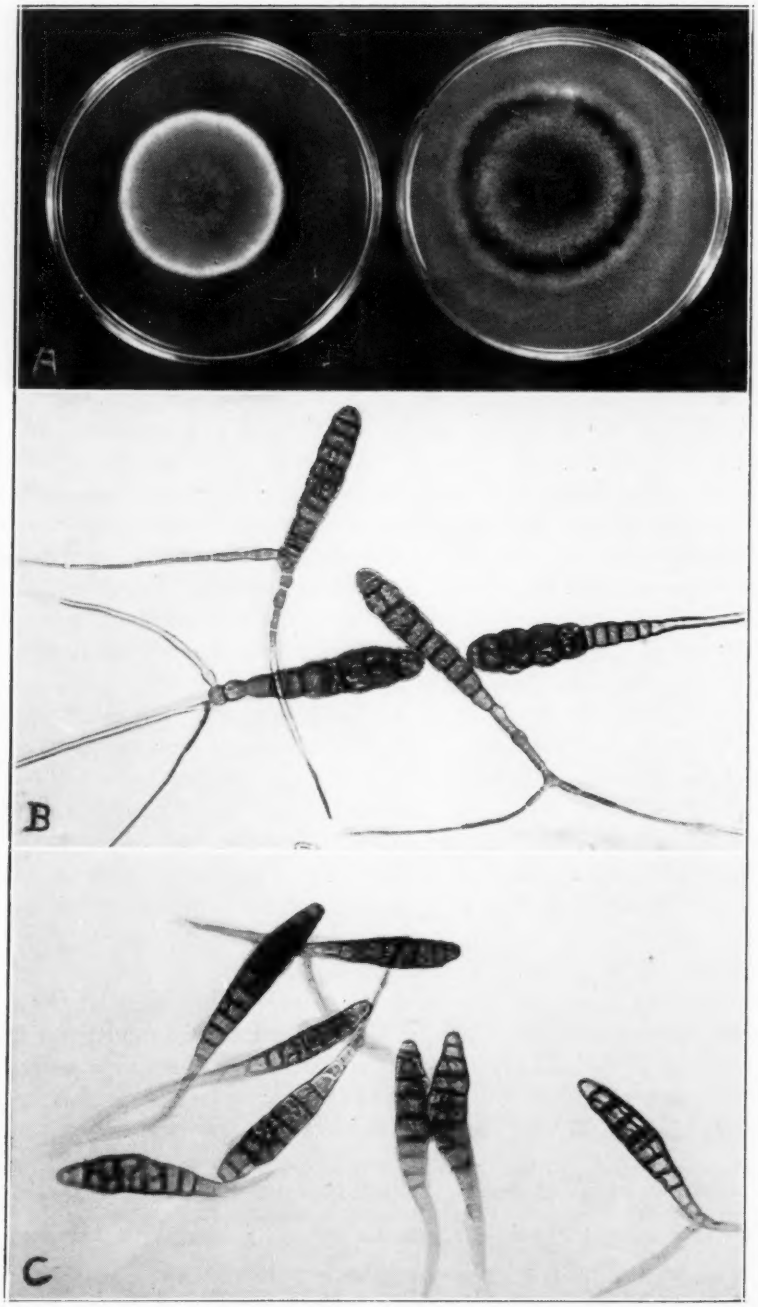

FIG. 1. A. Five-day-old culture on potato-dextrose agar of variant S–16 at left, and of typical chromogenic, sparsely sporulating *Alternaria solani* isolate at right. B. Conidia from S–16 culture. C. Conidia from sparsely sporulating, typical *A. solani* culture.

cultures obtained from these conidia were tested for pathogenicity by determining if they produced lesions at the base of the stems of young tomato seedlings. Only those cultures that were chromogenic were found pathogenic. No culture approaching S–16 in the production of conidia was isolated from the field. Variants have arisen frequently in *A. solani* cultures but no attempt was made in this work to segregate and study them. To date it has been possible to maintain S–16 as a nochromogenic, sporulating culture by mass transfer.

On potato-dextrose agar, S–16 grows more rapidly, has a darker surface color, and has a more diffuse type of growth than the usual isolate. Conidia may develop on 3-day-old cultures and on mycelium that is only 24 hours old. Plate cultures 8 to 10 days old have yielded the maximum number of spores.

Adequate comparison of the conidia from S–16 and with those from typical cultures have not been made because of insufficient numbers of conidia from the chromogenic cultures. In limited observations conidia from the S–16 culture have appeared somewhat the larger in culture. The following measurements of conidium length do not include the beak, as the length of the beak differs greatly in conidia that are of approximately the same size otherwise. Of 25 conidia of S–16 obtained on artificially inoculated tomatoes in the field, the mean length was $72.2 \pm 2.0\ \mu$ and the mean width was $17.7 \pm 1.9\ \mu$. The mean lengths of 3 samples of 100 *Alternaria solani* conidia, collected on naturally infected tomatoes in the field, were $83.3 \pm 1.0\ \mu$, $80.5 \pm 1.8\ \mu$, and $94.4 \pm 1.4\ \mu$. The mean widths varied from $17.3 \pm 0.3\ \mu$, $16.8 \pm 0.3\ \mu$, and $17.0 \pm 0.3\ \mu$. Since the different collections of *A. solani* conidia may vary considerably in size, it would seem that the S–16 culture probably is not significantly differentiated in size from the general population.

Collections of *Alternaria solani* conidia obtained in nature vary greatly in the proportionate occurrence of those with forked beaks. In 3 collections of conidia of *A. solani* studied, 2 were found to contain no fork-beaked conidia in 100 observed, while in the third collection 62 per cent were fork-beaked. In 100 conidia of variant S–16 from culture, 61 per cent were fork-beaked, while none of 25 conidia from the field were fork-beaked.

The pathogenicity of a conidial suspension of variant S–16 from culture was compared with that of a suspension of conidia of *Alternaria solani* collected in the field. A dilute suspension of conidia was placed in drops on tomato foliage after which the plants were kept moist for 72 hours. Infection occurred at all points of inoculation with each conidial suspension. No difference in the size of the leaf spots could be observed. The S–16 culture has produced stem lesions, leaf spots, stem-end rot of the fruit, and spotting of the green fruit in the field, similar to that produced by typical *A. solani*. In the field the S–16 variant has not been observed to sporulate more abundantly than other isolates of *A. solani*.

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CHLAMYDOSPORE GERMINATION IN THE FUNGUS CAUSING DWARF BUNT OF WHEAT[1]

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(Accepted for publication January 12, 1943)

INTRODUCTION

Dwarf bunt of wheat, caused by a peculiar race of *Tilletia tritici* (Bjerk.) Wint., is distinguished from the more common races of *Tilletia* spp. on wheat by certain striking symptoms and characteristics.[3] One of these is the apparent nonviability of the chlamydospores of the fungus under laboratory conditions, which also is reflected in the failure to obtain infection by seed inoculation. Recently it was reported[3] that certain hyaline, haploid cells associated with the chlamydospores were capable of germination, but it was pointed out that these viable cells were not present in sufficient numbers to perpetuate the disease in destructive proportions, unless the mycelium produced by them had unusual capacity for vegetative propagation in the soil. However, this possibility has been eliminated by the results of experiments (not yet reported) proving that the mycelium of the dwarf bunt fungus develops perhaps even less extensively in soil than does that of the ordinary bunt fungi. Therefore, further attempts to germinate the chlamydospores have been made, and the results are reported in this paper.

MATERIAL AND METHODS

The chlamydospores of the dwarf-bunt fungus used in these tests were collected from time to time in commercial wheat fields in 13 areas of infestation in the western wheat region. The spores of the various collections were stored at room temperature from 1 to 6 years, depending upon when the collections were made. All germination tests reported were made in 1942.

As already reported,[3] the various treatments used in earlier experiments on the dwarf-bunt chlamydospores failed to induce germination. In more recent experiments, however, the spores were soaked in tap water[4] for 1 to 7 months while stored in an electric refrigerator kept at about 4° C. The water was changed about once a month to reduce contamination.

Germination tests were made on 2 per cent water agar in Petri dishes. The period of incubation extended to 2 months at 3 temperatures approximating 5, 10, and 20° C. Observations to determine whether there was spore germination were made weekly, beginning with the first week of incubation.

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture, and the Washington Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[3] Holton, C. S. Preliminary investigations on dwarf bunt of wheat. Phytopath. 31: 74–82. 1941.

[4] Suggested by the fact that G. W. Fischer observed germination of apparently nonviable spores of certain species of *Tilletia* from grasses after the spores had been soaked in water one month.

RESULTS

The chlamydospores of all the collections tested germinated in percentages ranging from a trace to as high as 30 per cent, the highest percentages occurring at 10° C.

Perhaps more notable than the percentage of germination was the characteristically branched type of promycelium that was produced by the germinating spores (Fig. 1). The number of branches varied from 2 to 4 and the branching occurred at various points on the promycelium from the base (Fig. 1, J) to the tip (Fig. 1, E and F). Branches may arise laterally on the main promycelium (Fig. 1, C, I, and H) or else the main promycelium may divide at the tip and produce branches of similar dimensions (Fig. 1,

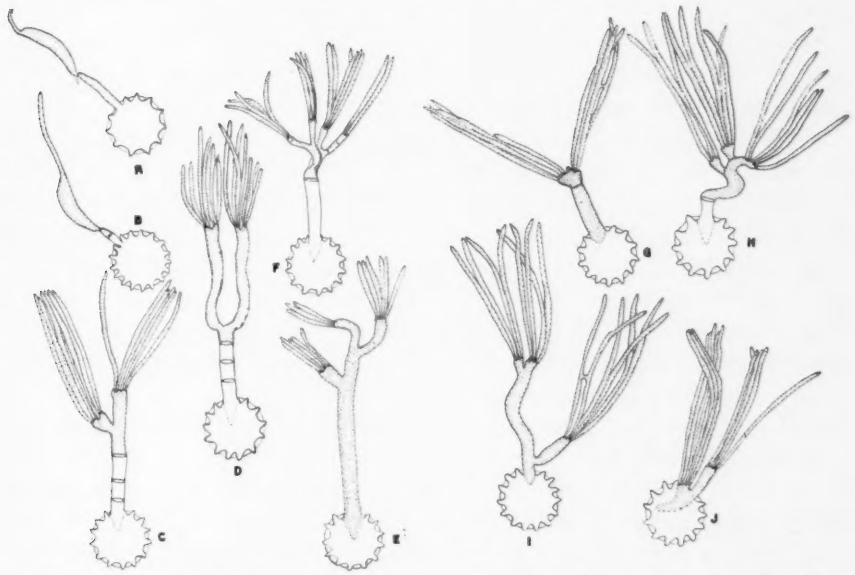

FIG. 1. Freehand drawings of germinating chlamydospores of the dwarf-bunt fungus. A and B. Haploid type of germination. C, H, and I. Lateral branches produced from the main promycelium. D. The main promycelium divided at the tip to produce two branches of similar dimensions. E. The main promycelium produced a lateral branch and then divided at the tip to produce two more branches. F. The promycelium divided at the tip resulting in two branches, each of which also divided at the tip, thus forming four branches. G. Two distinct groups of sporidia produced on the enlarged end of the promycelium. J. The main promycelium produced a lateral branch from its base. (Note the slight degree of bifurcation at the tips of branches in C, D, and I.)

D). Some of the branches appear to be septate (Fig. 1, F). Less frequently observed is the type shown in figure 1, G, in which two distinct groups of primary sporidia are borne on the enlarged end of the promycelium. Although not illustrated, unbranched promycelia were produced by many of the spores.

The germination process illustrated in figure 1, A and B, is similar to that already described for the hyaline cells.[5] Instead of a promycelium a

[5] See footnote 3.

sterigma is produced bearing a so-called secondary or sickle-shape sporidium, thus indicating that these chlamydospores are haploid. While this type of germination does not occur commonly, it has been observed on numerous occasions, suggesting that the presence of a limited number of these spores in dwarf-bunt collections may be regarded as typical.

The number of primary sporidia produced on each promycelial branch ranged from 2 to 8 (Fig. 1, F and J), and the total on any one promycelium ranged from 9 to 16 (Fig. 1, G and F). The length of the sporidia on certain promycelia varied considerably (Fig. 1, D and G), but whether this was due to different stages of maturity or to some other factor could not be determined. The primary sporidia fused in pairs (Fig. 1, G and I) in the same manner as is characteristic of the species. Apparently, most of the sporidia illustrated in figure 1 had not yet developed to the fusion stage. Unlike those of other races of the bunt fungi, the fused sporidia (H-shape structures) of the dwarf-bunt fungus rarely produced secondary sporidia. Instead, a mycelium was produced directly from the fusion. It is assumed that this mycelium was the infection hypha. On the other hand, secondary sporidia were produced in abundance in culture by certain of the monosporidial lines derived from the germinating hyaline spores,[6] thus indicating genetic differences for this behavior. The type of structures produced by the germinating spores may have some bearing on the classification of the dwarf-bunt fungus.

Whether the differences in viability of spores of different collections were due to inherent factors or to environmental influence has not yet been determined. However, in certain collections the percentage germination increased with prolonged soaking. For example, spores of one collection that had soaked 11 weeks germinated less than 5 per cent after 18 days' incubation at 10° C. After soaking for 4 months, these spores germinated 15 per cent in 8 days and after soaking 5 months they germinated 25 per cent in 4 days. Similar results were obtained with the spores of several other collections, whereas still other collections failed to respond to increased periods of soaking. However, the amount and degree of branching of the promycelium appeared to be reduced in spores that had soaked 5 months or more. Thus, while there were definite indications that increased periods of soaking increased the percentage germination, such increased soaking seemed to decrease the amount and degree of branching.

Whether the facts revealed by these observations on germination of dwarf-bunt chlamydospores are significant in relation to the natural occurrence of this disease would seem to be questionable, since, under field conditions, the spores are not subjected to a prolonged period of soaking in water immediately prior to the time when infection occurs. Under field conditions the spores, as well as the soil, are subjected to alternate wetting and drying and freezing and thawing, but these conditions in the laboratory failed to

[6] See footnote 3.

induce germination.[7] Nevertheless, the demonstrated fact that dwarf-bunt chlamydospores are viable and can be induced to germinate by a relatively simple method undoubtedly will lead to more effective investigation of this problem.

SUMMARY

Chlamydospores of the dwarf-bunt fungus were induced to germinate by prolonged soaking (3 months or more) in tap water kept at about 4° C. Germination was relatively low, ranging from a trace to about 30 per cent in different collections.

Most of the germinating spores produced a branched promycelium, the number of branches ranging from 2 to 4. Two to 8 primary sporidia were borne on each branch and some of these sporidia fused in pairs. A fused pair of sporidia usually produced a mycelium, which presumably was the infection hypha. Relatively few sickle-shape secondary sporidia were developed.

The type of structures produced by the germinating spores may have some bearing on the classification of the dwarf-bunt fungus.

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[7] See footnote 3.

CONTROL OF PITHIUM ROOT ROT OF ALOE VARIEGATA BY HOT-WATER TREATMENT

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(Accepted for publication November 20, 1942)

INTRODUCTION

In a local nursery several thousand seedling plants of *Aloe variegata* L. were discarded because of a root rot induced by *Pythium ultimum* Trow.[1] and possibly other Pythiaceae. The decay usually began at the root tip, and in a short time progressed back to the stem. Under commercial conditions of growth the final result was a stunted top with pallid, hard leaves and no roots (Fig. 1, A, B). However, if plants were kept quite moist the fungus advanced into the stem and leaves, reducing them to a soft, watery

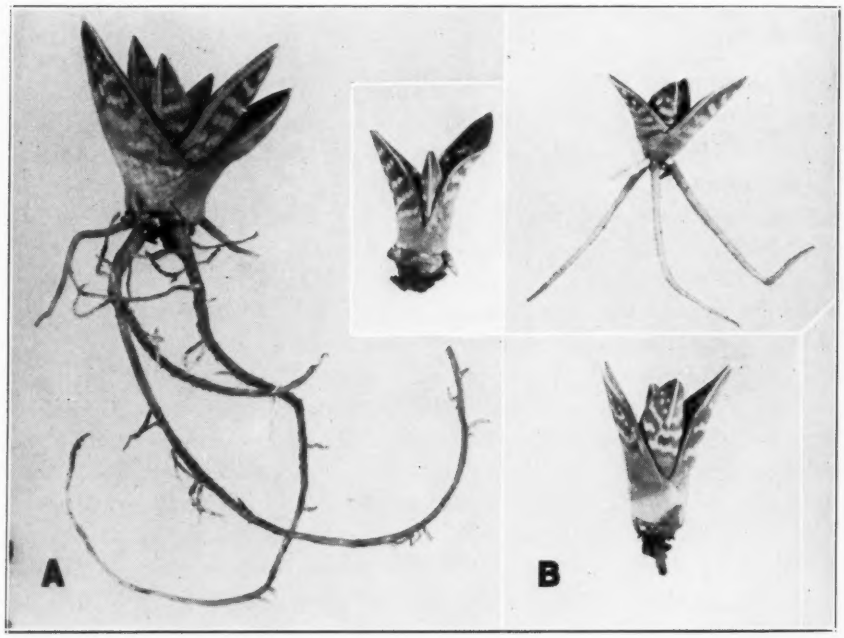

FIG. 1. Control of Pythium root rot of *Aloe variegata* by hot-water treatment at 46° C. for 20 minutes. A. Treated plant at left and check at right, 100 days after planting. B. Treated plant above, and check below, 20 days after planting; the former showing rapid recovery.

mass inside of the heavy epidermis, and soon decayed the whole plant. Since 2 to 3 years are required to produce plants for the market, the loss is considerable under the prevalent practice of discarding diseased seedlings.

The plant withstands relatively high temperatures in its native habitat in Karoo and Namaqualand, South Africa. This fact together with the

[1] Identified by John T. Middleton.

relatively low thermal tolerance of most Pythiaceae, suggested a method of heat treatment to salvage plants with decayed root systems.

The use of heat to destroy pathogens in living plant tissue was introduced 61 years ago by Jensen (4) when he treated seed-potato tubers in a water-jacketed chamber for 4 hours at 40° C. to destroy the internal mycelium of *Phytophthora infestans* (Mont.) de Bary. This apparently was not practiced on a field scale, and Jones, *et al.* (5) later found no practical field benefits from its use in Vermont. Several subsequent investigators have used similar treatments against other Pythiaceae in living vegetative structures. Rosenbaum (9) found that immersion of green tomato fruits in water at 60° C. for 1½ minutes killed the mycelium of *Phytophthora parasitica* Dast. (*P. terrestris* Sherb.) in non-visible surface lesions. Fawcett (2) was able to destroy the mycelium of *P. citrophthora* (Sm. and Sm.) Leon. in lemon fruits by holding them in hot water for 2 minutes at 48.9° C. within 30 hours after infection, or at 46.1° C. within 8 hours after infection. In 1926 Murphy and McKay (6) found that dry air at 40° C. for 8, 16, or 24 hours, or at 38° C. for 16 hours killed the mycelium of *Peronospora schleideni* Unger without injury to onion bulbs. Brown (3) reported good control of crown and root rot of peony after removal of diseased areas and treatment in water at 48.9° C. for 30 minutes; *Phytophthora cactorum* (L. and C.) Schroet. (*P. paeoniae* C. and P.) was among the organisms involved. Ark and Barrett (1) treated asparagus spears infected with *Phytophthora* sp., killing the organism by immersion in hot water at 46° C. for 1 minute.

METHODS AND RESULTS

In this study a total of 505 plants of *Aloe variegata*, 1, 2, and 3 years old, were dried for several days in the laboratory and then immersed in water of varying temperatures to determine the heat sensitivity of the host and parasite. The plants were those actually discarded because of root rot by the nurseryman and were selected only to the extent of eliminating a few with rotted tops. Wire test-tube baskets with cheesecloth tops were used to hold the seedlings during immersion. After treatment the plants were spread out to cool and dry before being planted in pasteurized light sandy loam; the checks were planted in other containers of the same soil. Some were planted singly in 3-inch pots and some spaced about 1½ inches apart in flats. The soil was kept very wet and the plants held in a cool, humid greenhouse, conditions most favorable for the development of the disease and apparently so recognized by growers (7, 8).

Three tests were made at different times using, variously, water at 40.5, 43, 46, 49, 51.5, 54.5 and 57.5 degrees C. for 20 minutes, 46° C. for 30 minutes, and 46° and 49° C. for 40 minutes. Temperatures lower than 46° did not consistently kill the pathogen, and those of 49° C. and above frequently were injurious to the Aloes. The treatment at 46° for 20 or 30 minutes eradicated the fungus without injury to the host, but the 40 minute period produced some subsequent drying of the outer leaves and might be

regarded as excessively damaging. The treated plants very quickly formed new roots which in 20 days were in some cases 2 inches long, whereas the checks had been unable to produce new roots (Fig. 1, B). The difference was even more pronounced after 100 days (Fig. 1, A).

Under commercial conditions, the use of a water treatment at 46° C. for 30 minutes would be advisable, but this may be reduced to 20 minutes for very small plants. If large specimens, such as seed-bearing plants, are to be treated the time might be extended safely to 40 minutes. The precaution of replanting the seedlings in soil freed of the pathogen by pasteurization or other means should be observed.

Limited tests with *Haworthia attenuata* Haw. gave results comparable to the above. Indeed, it seems probable that many of the cacti and succulent plants would tolerate the treatment and that salvage of plants now discarded might be economically effected.

SUMMARY

Pythium ultimum causes an important root rot of young nursery plants of *Aloe variegata* in California. Because heat treatment of the plants will free them of the pathogen, the prevalent costly practice of discarding infected plants is unnecessary. A hot-water treatment of infected plants at 46° C. for 20 to 40 minutes, depending on the size of the plants, kills the parasite without injury to the host. It is essential that treated material be replanted in soil free of the pathogen.

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PHYTOPATHOLOGICAL NOTES

The Production of Coacervates.—Coacervates of organic compounds often characterize pathological conditions in plant cells. The coacervates generally consist of refringent, spherical masses of phenolic material, each with a lipoidic envelope. It seems probable that these bodies are formed as a result of the disturbance of hydrogen bonds in the catechol-water system by the activity of a catechol oxidase. Dufrenoy and Reed[1] showed that the main factor in the production of the auto-complex coacervation of a phosphatid is a certain stage of desolvation. The presence of catechol or other polyphenol dispersed in the cell vacuole may act as a desolvant agent.

This note will discuss the results of experiments *in vitro* on coacervate production with chemicals. Lecithin was stained in a solution of Sudan III in methylal to make it identifiable under the microscope.

The methylal solution of Sudan III has proved to be an exceedingly useful reagent for staining the above mentioned spherical inclusions in the cell vacuole, following the technique earlier given by Dufrenoy and Reed.[2] A few cc. of this lecithin-Sudan III solution were added to the surface of a solution of catechol in a buffer solution having a reaction of pH 5.35. At the end of three hours, it was evident that the lecithin had assumed a finely divided condition. Under the microscope, one could see many globular masses, resembling coacervates (Fig. 1), each enveloped in a vesiculated

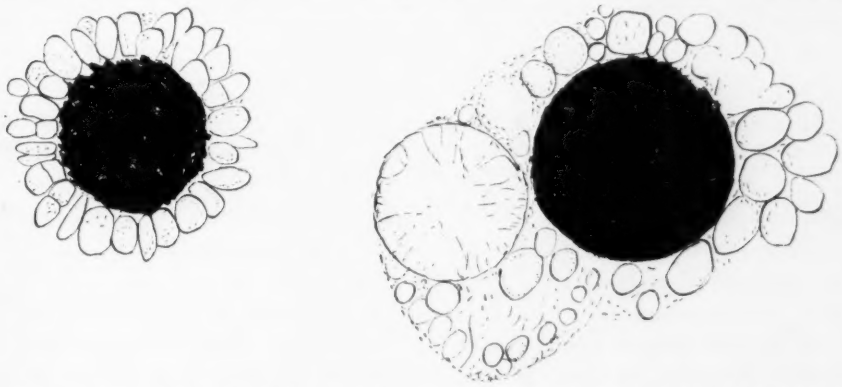

FIG. 1. Coacervates of catechol, each surrounded by a vesiculate envelope of lecithin.

layer of lecithin (identified by its orange-red color). De Jong and Hartcamp[3] observed similar hyaline vesicles on Paramecia, which had been exposed to certain chemicals that affected the solvation of the cell constituents.

The vacuolate nature of the lipoid envelope, which contains water as well as lecithin, was demonstrated in hypoplastic plant cells of orange roots that had grown in a solution lacking micro-elements.[4] Similar coacervates were

[1] Dufrenoy, J., and H. S. Reed. Coacervates in physical and biological systems. Phytopath. 32: 568–579. 1942.

[2] ————, and ————. A technic for staining cells with Sudan III in a water phase. Stain Tech. 12: 71–72. 1937.

formed in solutions of catechol buffered at pH 6.35 and pH 7.35, though smaller, and often with radiating strands of lecithin. The recent discovery that plants suffering a deficiency of micro-elements may contain coacervates of pyridoxin[5] gives point to the investigation of pyridoxin-lecithin complexes *in vitro*. The photomicrographs (Fig. 2) show coacervates produced in

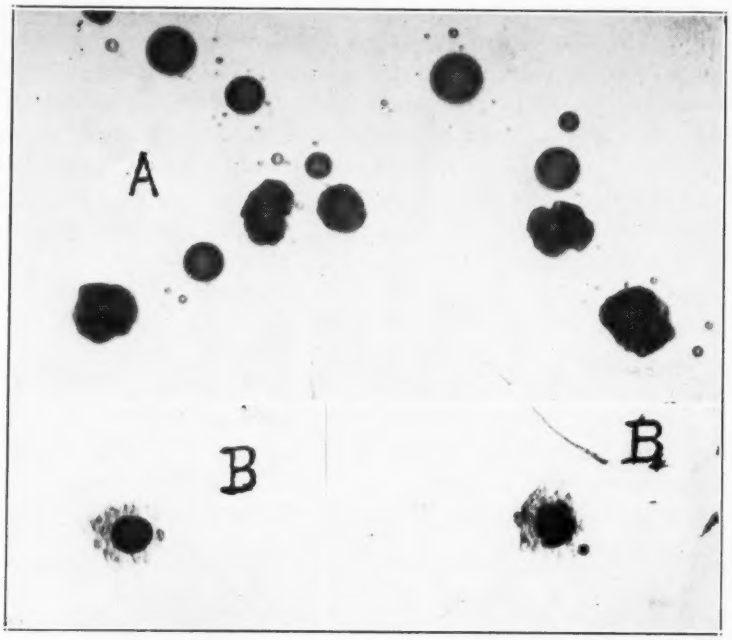

FIG. 2. Photomicrographs of coacervates of pyridoxin with lecithin, ×1290. A. Coalescence into auto-complex coacervates was evident. B. Vesiculate envelopes of lecithin surrounded the larger masses. Photographs by Dr. R. M. Reeve.

buffer solutions at pH 5.35 by the technique above described. The tendency for small coacervates to coalesce and to form larger ones, observable *in vivo*, is seen in the illustration.

I experimented also with resorcinol and hydroquinone. Coacervates were obtained with resorcinol dissolved in water, but when dissolved in a phosphate buffer solution, it did not form coacervates with lecithin. Solutions having a pH of 5.35 and 7.35 were tried, but yielded only droplets of red-stained oil, surrounded with halos of vacuolated, hydrated lecithin. In this case the resorcinol was probably not sufficiently oxidized to change the electric charge on the micelles of lecithin. Hydroquinone did not produce real auto-complex coacervates when 2 per cent solutions were placed in contact with methylal containing lecithin. In a phosphate buffer of pH 7.35 the solution turned brown after 2 days, but yielded no agglomerations resembling coacervates.

I repeated the experiments, using different solvents for lecithin, namely, chloroform and toluol, without obtaining coacervates. Good results were

obtained, however, by using alcohol as a lecithin solvent and gently placing a layer of it on top of a solution of catechol + neutral red in a phosphate buffer at pH 5.35. The alcohol solution mixed slowly with the aqueous system and allowed time for desolvation and coacervation.

The relation of coacervates to health and disease has been discussed in the works here cited. This note describes aggregates that were produced with chemicals in a simple system *in vitro*, thereby contributing information on the formation of coacervates and certain intra-cellular activities.—HOWARD S. REED, University of California, Berkeley, California.

Breaking in Color of Flowers of Annual Phlox Caused by the Aster-yellows Virus.—In 1938 one annual phlox plant (*Phlox drummondii*) showing breaking in color of the flowers was found on the campus of the University of California at Berkeley. All attempts to transmit the virus from diseased to healthy annual phlox grown from seeds by juice inoculations using the carborundum method were failures.

It was suspected that the virus causing the breaking in phlox was celery calico, which commonly induces breaking in pansies and violas in California. The virus extract from a number of host plants of celery calico inoculated in phlox failed to induce breaking. This virus is transmitted by 10 species of aphids, but all aphid inoculations to healthy phlox were negative.

Another virus that causes breaking in the color of pansies, violas, petunia, and ranunculus is western cucumber mosaic, which occurs in the interior regions of California. Juice and aphid inoculations failed to induce breaking in the color of annual phlox, even though it was infected with this virus. The flowers were dwarfed with rolled petals, and sometimes the corollas were dead.

In 1941 many ornamental flowering plants grown in the canyons of the Montara Mountains, where the cut-flower trade is an important industry, were affected with aster yellows. Five acres of strawflowers were destroyed by his disease. Annual phlox manifested both breaking in the petals and symptoms of aster yellows.

Lots of 20 previously noninfective, short-wing and long-wing aster leaf hoppers, *Macrosteles divisus* (Uhl.), after feeding for a few days on diseased phlox removed from the field, were transferred and kept on healthy asters that developed typical symptoms of aster yellows. It is evident from this experiment that phlox was naturally infected with aster yellows.

Attempts were made to infect annual phlox, grown from seed, with the California aster-yellows virus, which is not transmissible to any host plant by means of juice inoculation at room temperature; hence, infective aster

[3] Jong, H. G., Bungenberg de, and J. L. Harteamp. On the formation of hyaline vesicles at the surface of *Paramecium caudatum*. Protoplasma. 31: 550–587. 1938.

[4] See footnote 1.

[5] Reed, H. S., and Jean Dufrenoy. Pyridoxin and coacervates in plant cells. Science 96: 470. 1942.

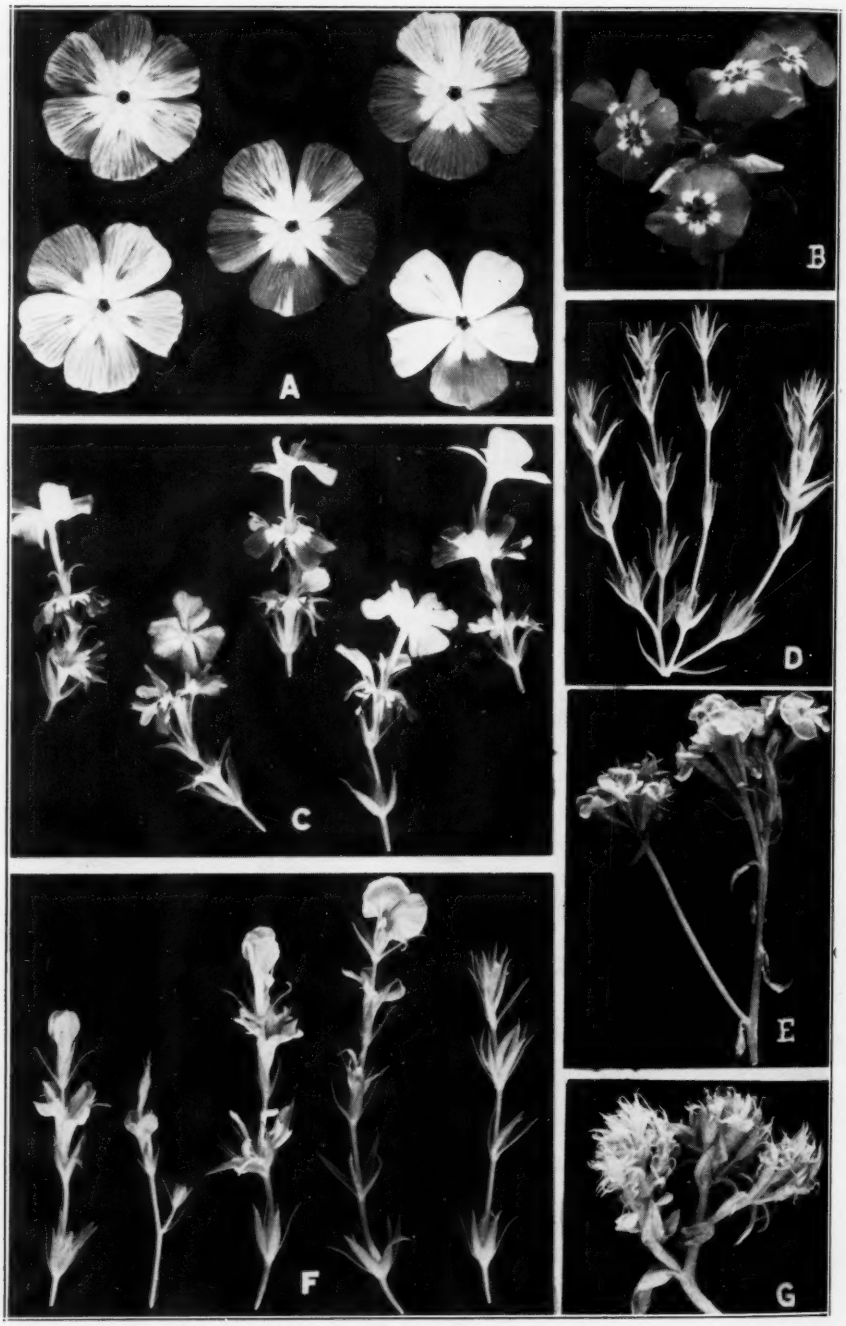

FIG. 1. Symptoms of California aster yellows on annual phlox (*Phlox drummondii*). A. Breaking on color of petals. B. Cluster of flowers from healthy check, or control plant. C. Proliferation of flowers showing breaking in color of petals. D. Proliferation and virescence or greening of flowers, the petals being reduced to leafy structures resembling sepals. E. Virescence of petals. F. Proliferation of flowers with green leafy petals on lower flowers and dwarfed apical flowers with one or more petals absent. G. Cluster of green flowers with no proliferation.

leaf hoppers were used. Healthy phlox plants were exposed 3 weeks or longer to 20 infective male short-wing aster leaf hoppers that had completed the nymphal stages on diseased celery plants or 20 infective male long-wing aster leaf hoppers that had completed the nymphal stages on aster-yellows plants. Males were used rather than females to avoid egg deposition. The males were then removed, and the inoculated plants and the check or control plants were put in an insect-proof cage until the blossoming period. The aster-yellows virus induced breaking in the petals of phlox (Fig. 1, A). The virus was recovered by previously noninfective short-wing and long-wing aster leaf hoppers and transferred to healthy aster and celery plants, respectively.

Breaking in color of the petals consists of white veinbanding, sometimes one or more petals show breaking and the remainder are white with a few streaks of the normal color of the flower (Fig. 1, A). Frequently, virescence or greening of the petals occurs (Fig. 1, E) without breaking. Proliferation of the flowers occurs, successive flowers developing from the ovaries. Breaking often is evident on successive proliferated flowers (Fig. 1, C). In the advanced stage of the disease proliferation and virescence of the flowers are common on infected plants, the petals being reduced to green leafy structures resembling sepals (Fig. 1, D), sometimes, with a dwarfed apical flower with one or more petals absent (Fig. 1, F). Frequently, clusters of green flowers develop with no proliferation of flowers (Fig. 1, G).

It is interesting to note, that this is the first case of a leaf-hopper-transmitted virus inducing breaking in color of flowers.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, Berkeley, California.

An Unusual Bean Disease.[1]—An apparently new disease of beans has been observed in field and garden beans grown for seed in southern Idaho. The disease is characterized by a pronounced reddish discoloration of the nodes, which usually is the first indication of its presence. In the later stages the leaves become malformed and the veins are of a decided reddish color (Fig. 1, A). Reddish colored sunken lesions are usually, though not always present on the pods of plants having red nodes and veins (Fig. 1, B).

The disease does not kill the plant, but causes dwarfing, a reduction in the number of pods produced, and premature ripening. When seeds are removed from ripe diseased pods similar to those shown in figure 1, B, they are often severely marked with target-like spots or concentric lines on the seed coats (Fig. 1, C). The seeds from such pods are usually small and often shrivelled.

The disease is more severe in some varieties than in others. The variety Bountiful is particularly susceptible. Great Northern and Red Mexican field beans are also very susceptible, although the disease does not mark the

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Researeh Paper No. 217.

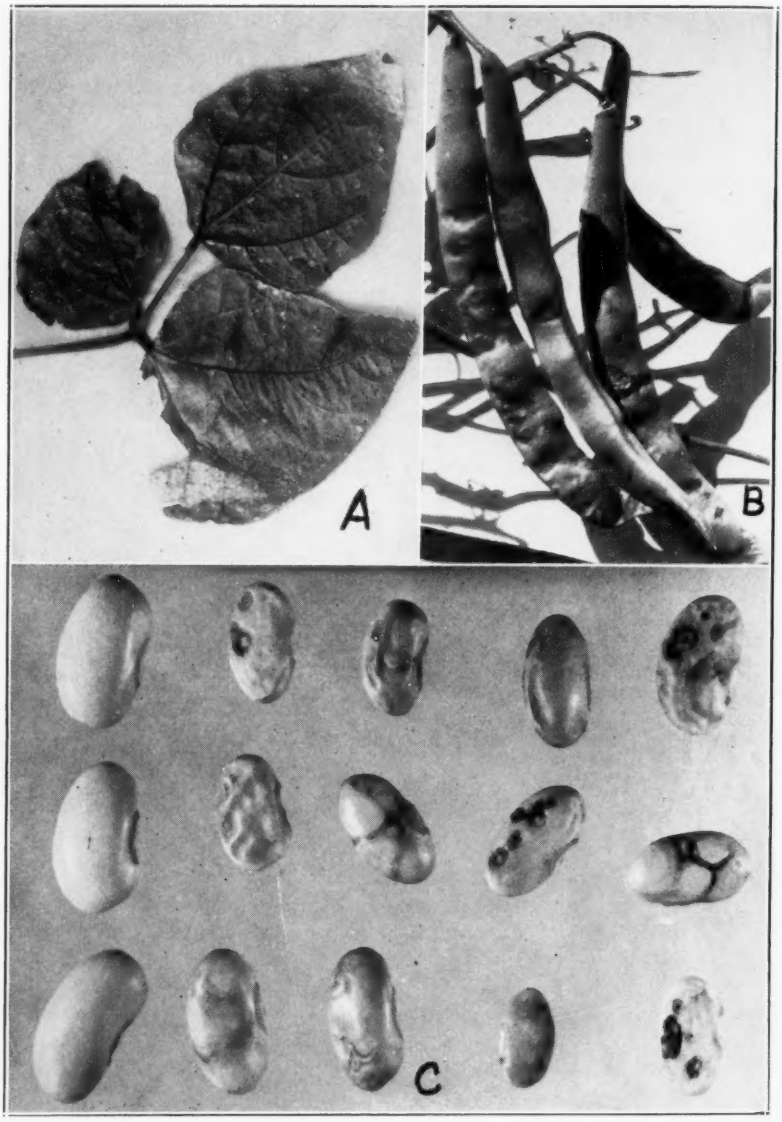

FIG. 1. A. Reddish discoloration of the veins. B. Pods with severe lesions. C. Seeds from infected pods, similar to those shown in B.

seed so severely. The disease has been found to be more prevalent along the edges of bean fields.

Preliminary evidence indicates that the disease is caused by a virus, and appears to be closely related to bean virus 2, or yellow bean mosaic virus.

Affected seeds similar to those shown in figure 1, C, were planted in pots in the greenhouse. Most of the seeds germinated. Plants from 48 seeds were grown to maturity and no sign of the disease was visible on any of the

plants. This would indicate that the disease is not seed borne.—W. J. VIRGIN, Idaho Agricultural Experiment Station, Moscow, Idaho.

A Leaf Disease of Kentucky Bluegrass.[1]—In cool, wet periods, particularly in the spring, whole fields and lawns of Kentucky bluegrass, *Poa pratensis*, in central Kentucky may take on a yellowish-brown cast as a result of a leaf disease. This is usually at a time when growing conditions should be about ideal. The same disease also may appear in the fall when it may again cause extensive injury to the foliage. Injury appears to occur first on the leaf sheath, and may spread through the sheath to young leaves or tissues within, eventually killing all leaves of a shoot. The blades of leaves affected on the sheath often begin to yellow at the tips, as well as the base, and finally turn completely yellow and die. Under somewhat drier conditions small dark-brown spots may be seen on leaf blades and sheaths. During the summer new infections are rare. The disease rarely kills plants, but may cause nearly complete defoliation.

In 1940 and since that time, isolations from affected tissue have fairly consistently yielded an organism that J. R. Hardison has recently identified as *Septoria oudemansii* Sacc. Pycnidia of this fungus are commonly found on dead leaf blades. They contain spores that appear to be non-septate, or occasionally 1- or 2-septate, hyaline, and measure about 2.5×14.5 μ. In culture on potato-dextrose agar at 15° C. black stroma-like bodies were produced in which the pycnidia were immersed. On sterilized bluegrass leaves pycnidia were produced in abundance at 25° C. Sections of infected leaf tissue stained with cotton-blue showed the fungus to be intercellular.

Septoria oudemansii has been reported on *Poa pratensis* as a leaf spot in Michigan by Hardison,[2] in Oregon by Sprague,[3] and in Western United States by Fischer *et al.*;[4] but the importance of this fungus as a cause of a serious disease of bluegrass seems not to have been recognized heretofore.—ROBERT B. GRIFFITH, Kentucky Agricultural Experiment Station, Lexington, Ky.

Powdery Mildew on Ribbon-bush (Homalocladium platycladum Bailey).—The attention of the writer was recently called to a powdery mildew affecting a plant of the ribbon-bush (*Homalocladium platycladum* Bailey), a native of the Solomon Islands. The plant, a member of the Polygonaceae, is unusual in possessing flattened, strap-like stems (phyllodia), little thicker than the leaves. The leaves are borne singly at the nodes, with their flat surfaces in the same plane as the stem (Fig. 1). Mildew infection was heavy on the leaves, causing yellowing and distortion, but was absent from the stem

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Hardison, J. R. Grass diseases in Michigan in 1941. Plant Dis. Rptr. 26: 67–75, 1942.

[3] Sprague, Roderick. A revised check list of parasitic fungi on cereals and other grasses in Oregon. Plant Dis. Rptr., Supplement 134, 1942.

[4] Fischer, George W., Roderick Sprague, Howard W. Johnson, and John R. Hardison. Host and pathogen indices to the diseases observed on grasses in certain western states during 1941. Plant Dis. Rptr., Supplement 137, 1942.

tissues. Perithecia were abundant and proved to be those of a species of *Erysiphe*, probably *E. polygoni*. Mildew has been severe on the foliage of this plant each winter since it was obtained, some 4 or 5 years ago; but it is

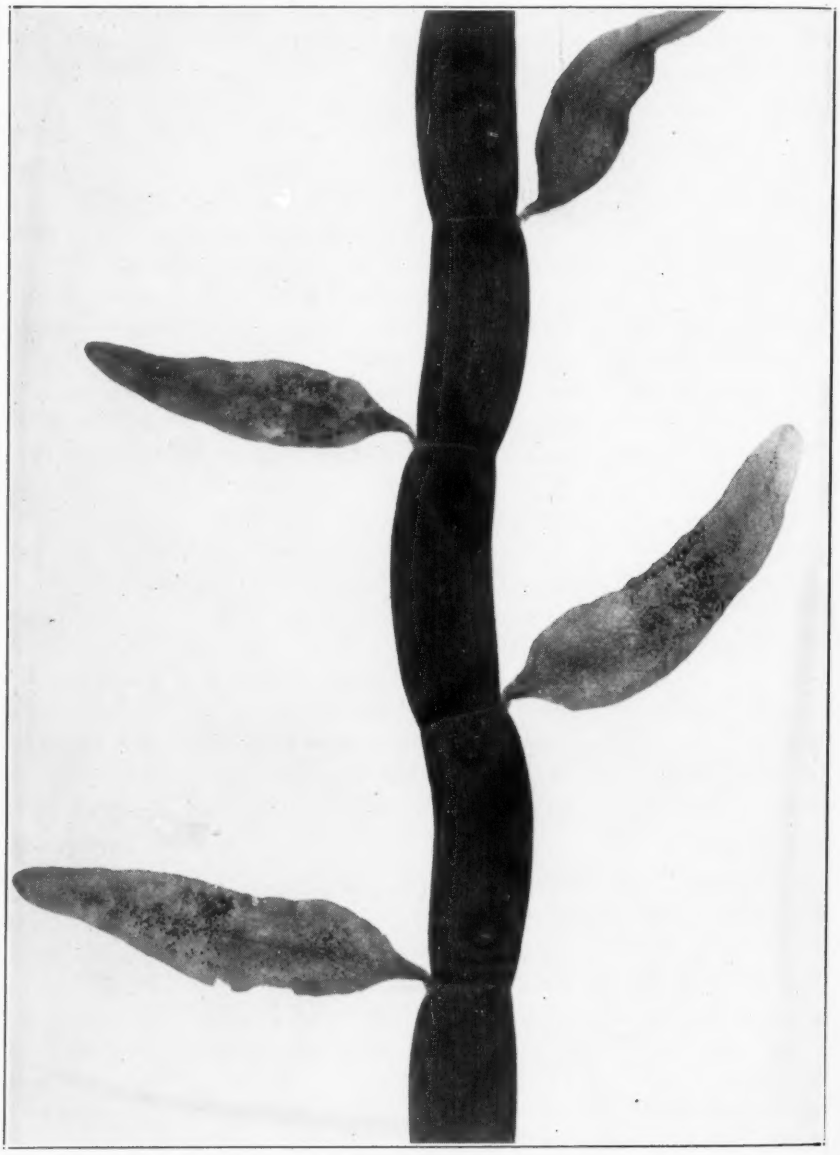

FIG. 1. *Erysiphe* sp. on *Homalocladium platycladum*. Note perithecia on true leaves and absence of infection from the phyllode or stem tissues.

not known whether the mildew was brought in with the plant or came over subsequently from some native host. The writer has been unable to find a previous report of powdery mildew on *H. platycladum*.—A. W. DIMOCK, Cornell University, Ithaca, New York.

REPORT OF THE WAR COMMITTEE

Reports of subcommittees indicate good progress all along the line. The regional committees have done an excellent job in compiling and publishing in mimeograph form essential facts about the prevalence and control of diseases of major importance in their respective areas. These are being put to good use in extension work throughout the country.

A development of outstanding significance is the recent announcement of the emergency expansion of the Plant Disease Survey in which 24 full-time plant pathologists will be utilized. This should be a potent factor in safeguarding our food supply. It gives us an opportunity to prove the value of the Survey, but it also places a real responsibility upon us to see that this emergency survey is effectively carried out. This is a responsibility, not only of Dr. Edson and his staff, but of each and every member of our Society.

The fungicide subcommittee is again sponsoring cooperative studies on substitute fungicides. One of the most outstanding results of these studies so far is the promising value of a number of organic seed protectants. These appear to give good results on practically all vegetable seeds, and seem to have a wide latitude in dosage without seed injury. Such fungicides should overcome many of the difficulties in the way of pretreatment of packet seeds. Dr. George L. McNew has been assigned the task of investigating the possibility of getting seed dealers to pretreat more of their packet seeds.

The executive committee has spent considerable time on the manpower situation. After a number of conferences the War Committee of the Economic Entomologists joined with this committee in requesting that a national committee on Plant Pathologists and Economic Entomologists be established to advise the National Roster and the Selective Service on matters pertaining to deferment and to more effective use of trained personnel in these fields. Our suggestions were favorably received. The resolution prepared by the War Committee together with data obtained from the survey made by Dr. Keitt have been submitted as directed. At the request of the National Roster, the committee is compiling data on plant pathologists in the armed services for their use in obtaining more efficient use of highly trained personnel already in service.

A reply has been received from Dr. Noble Clark stating the Directors' policy relating to regional meetings. A copy of this letter has been sent to contact men for their guidance in arranging future regional meetings. The essential requirement is that the meeting be sponsored by a Director or the Chief of a Federal Bureau.

Plans are under way for a meeting of the Upper Mississippi Valley Regional Committee in August, at Lafayette, Indiana. It is tentatively

planned to have a meeting of the War Committee in conjunction with this regional meeting.

The Committee has acted favorably upon the suggestion that a subcommittee on Market Pathology be created. Dr. C. O. Bratley has been requested to act as chairman. Other members of the committee will be announced later.

Dr. Stakman has finally returned from his mission to Mexico and has resumed the committee chairmanship.

Respectfully submitted,
J. G. LEACH, Acting Chairman
July 1, 1943